JOURNAL OF DAIRY SCIENCE

Contents

Measurement of Butyric Acid in Fat with Reference to the Detection of Substitute Fats in Dairy Products. W. J. HARPER AND T. V. ARMSTRONG 481

Parenteral Administration of Aureomycin to Young Calves with a Note on Mode of Action. L. L. RUSOFF, J. M. FUSSELL, C. E. HYDE, R. M. CROWN, AND L. S. GALL 488

Apyrase Activity of the Mammary Gland. E. M. CRAINE AND R. G. HANSEN 498

Phosphokinase Activity of the Mammary Gland. E. M. CRAINE AND R. G. HANSEN 505

A High Roughage System for Raising Calves Based on Early Rumen Development. IV. Synthesis of Thiamine and Riboflavin in the Rumen as Influenced by the Ratio of Hay to Grain Fed and Initiation of Dry Feed Consumption. H. R. CONRAD AND J. W. HIBBS 512

Lactation Response to Artificially-Dehydrated and Pelleted Alfalfa Added to a Ration of Timothy-Grass Mixed Hay and Grain. F. S. WARREN, R. E. JOHNSON, CECILIA M. DEMBICZAK, H. D. EATON, GEOFFREY BEALL, AND L. A. MOORE 523

Aspartate Dissimilation Reactions of Rumen Bacteria. F. M. SIROTNAK, R. N. DOETSCH, ROSLYN Q. ROBINSON, AND J. C. SHAW 531

A Comparative Study of Human, Cow, Sow, and Rat Milk Using Paper Chromatography. H. R. ROBERTS, J. D. PETTINATI, AND WESLEY BUCEK 538

A Comparison of Some Cellular and Chemical Constituents of Blood at Time of Parturition and After Administration of Adrenocorticotrophin. W. G. MERRILL AND VEARL R. SMITH 546

A Simple Test of Quality in Hay. EMMETT BENNETT AND J. G. ARCHIBALD 552

New Advances in the Electroejaculation of the Bull. WILLIAM G. R. MARDEN 556

Outdoor Individual Portable Pens Compared with Conventional Housing for Raising Dairy Calves. L. R. DAVIS, K. M. AUTREY, H. HERLICH, AND G. E. HAWKINS, JR. 562

The Effect of Cation and Anion Resin Mixtures on the Salt Content of Raw Skimmilk. G. K. MURTHY AND R. MCL. WHITNEY 571

Studies on the Use of Permanent Milk Pipelines in Dairy Barns. III. Labor (Time) Requirements for Operation of Pipeline and Conventional Installations in a Stanchion Barn. W. O. NELSON, E. E. ORMISTON, AND M. H. ALEXANDER 583

Report of Committee on Butter 590

Program — Forty-ninth Annual Meeting of the American Dairy Science Association 593

People and Events 616

Letters to the Editor 621

Our Industry Today:

The Fortification of Whole Milk and Skimmilk. L. A. MAYNARD 624

The Self-Help Program. MACK GEBERT 628

A Filtration Method for Roller Process Dry Buttermilk Solids. D. R. STROBEL AND C. J. BABCOCK 631

Abstracts of Literature A65

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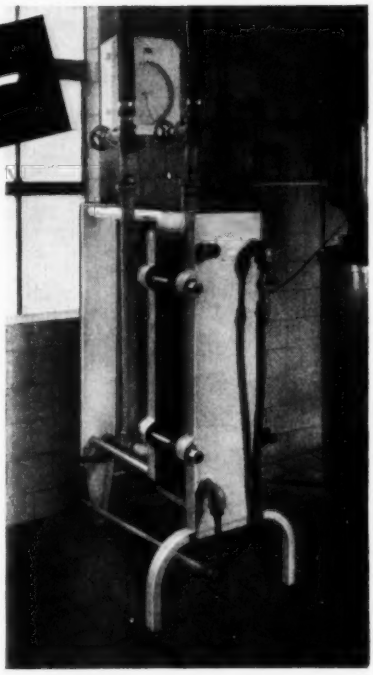

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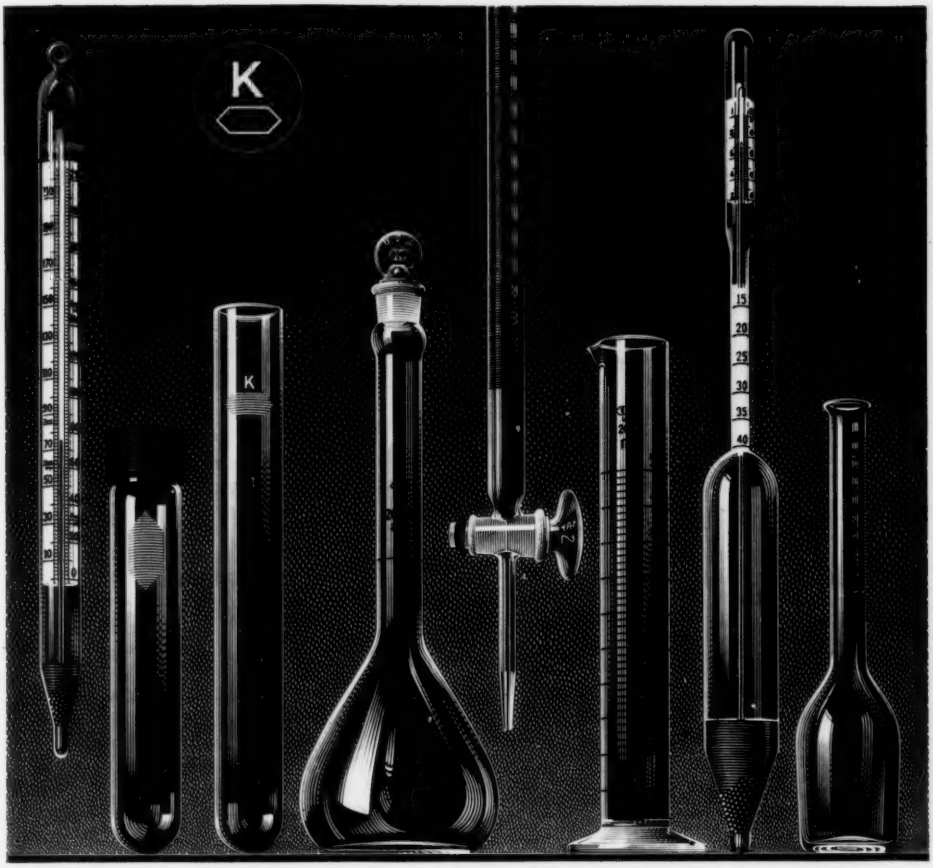

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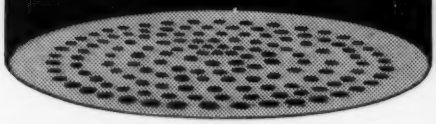

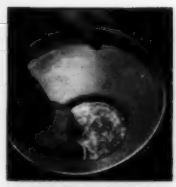

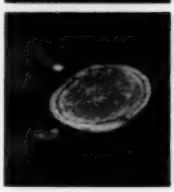

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MEASUREMENT OF BUTYRIC ACID IN FAT WITH REFERENCE TO THE DETECTION OF SUBSTITUTE FATS IN DAIRY PRODUCTS[1]

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Institute of Nutrition and Food Technology
The Ohio State University

Recently, there has been an increasing number of dairy products in which a part or all of the butterfat has been replaced by various vegetable and/or animal fats. The ease with which such products might be sold fraudulently as full butterfat products has emphasized the need for more precise methods of detecting fat mixtures. Older methods of fat analysis, such as the Reichert-Meissl, Polenski, and Hehner, are now thought to be inadequate in detecting mixtures of butterfat and other fats because they lack specificity and sensitivity. Consequently, a number of other methods have been prepared or are the subject of current investigations.

Methods recently reported involve the different properties of fat, such as fluorescence *(8)*; differences in solubilities *(2, 3)*; the tocopherol *(1)* or sterol content *(2)*; selective solidification *(7)*; refractive index *(9)*; certain fat constants *(3, 9)*, and butyric acid content *(6)*.

Any method which is to be satisfactory for the detection of fat substitution in dairy products must make possible the detection of both vegetable and animal fats. Since milk fat is the only natural fat which contains butyric acid, measuring this component appears to provide a satisfactory basis for detecting substitution of both animal and vegetable fat.

This investigation was undertaken to develop a relatively simple and rapid method for the routine measurement of the molar concentration of butyric acid in dairy products and to determine its value in detecting fat substitution.

EXPERIMENTAL

The chromatographic method used in this study was a modification of two procedures that were developed *(a)* to measure acetic, propionic and butyric acids in cheese *(4)* and *(b)* to study the free acids formed by lipolysis *(5)*. The method utilizes the principles of both partition and absorption chromatography

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and permits the direct saponification of the fat without extracting it from the dairy product prior to determining the molar concentration of butyric acid.

The method is outlined briefly as follows:

1. *Hydrolysis of sample:* (Ice Cream) 6 ml. of melted ice cream are measured into a 50-ml. beaker, and 1.0 ml. of saturated KOH and 13 ml. of 95% ethanol are added. The beaker is immersed in a boiling water bath for 15 minutes with occasional stirring. After hydrolysis, water is added to bring the volume to 20 ml.
2. *Preparation of reagents: (a)* Chloroform (U.S.P. or Technical Grade) is washed four times by shaking with water to remove any traces of ethanol. *(b)* 0.75 n-butanol in chloroform is prepared by adding 0.75 ml. of n-butanol to 99.25 ml. of washed chloroform. *(c)* 5.0% n-butanol in chloroform is prepared by adding 5 ml. of n-butanol to 95 ml. of washed chloroform. *(d)* Silicic acid (Mallinerodt No. 2847 specially prepared for chromatographic analysis) may be used directly after drying for 24 hours in a 100° C. oven. Faster analyses are obtained, however, by washing the silicic acid with water, allowing it to stand for 30 minutes and decanting the supernatant. Repetition of this procedure, followed by 48 hours of drying in a 100° C. oven removes the fine particles of silicic acid and provides adequate dryness of the silica gel. *(e)* The pH 6.4 buffer (2*M*) is prepared by mixing 2*M* KH_2PO_4 and 2*M* K_2HPO_4. *(f)* 0.01 *N* KOH is prepared by dispersion in absolute ethanol (isopropyl may be substituted). *(g)* Phenol red (0.1%) is prepared by grinding 100 mg. of phenol red with 0.1 ml. of *N* KOH and diluting to 100 ml. with absolute ethanol. Phenolphthalein (0.1%) in absolute alcohol may be used in place of phenol red.
3. *Preparation of buffered column:* A 35-mm. I.D. chromatographic column (38 mm. O.D. × 250 mm., with 34/28 T-joint containing a sealed perforated-fritted glass disk; Kauffman-Lattimer Cat. No. C-4084 III) is used; 12.5 g. of dry silicic acid and 6 ml. of pH 6.4 buffer are mixed with a pestle in a mortar and transferred to a 100 ml. beaker. Enough washed chloroform (30-40 ml.) is added to make a free flowing slurry, and this slurry is added to the column after a 34-mm. filter paper disc is placed on the perforated plate. A filter paper disk is then placed on top of the buffered part of the column. Enough chloroform is added to prevent the column from becoming dry, but no pressure is applied.
4. *Preparation of sample section:* Two ml. of the saponified sample is added to a mortar, then 0.8 ml. of 20% sulfuric acid is added, and the sample is mixed thoroughly. The acidified sample is then ground with a pestle with 5 g. of dry silicic acid until completely mixed, and the dry mixture is quantitatively transferred to a 50-ml. beaker. Thirty ml. of washed chloroform is added, and the resulting slurry is poured into the column on top of the buffered section when the upper level of the previously added chloroform has reached the top of the filter paper disk at the top of the buffered section. At this time the column is placed over an intermittent 150-ml. siphon, and collection of the higher fatty acids is started. The

beaker is then rinsed with two 10-ml. portions of washed chloroform to remove remaining acids. Fifty additional milliliters of washed chloroform is added to the column slowly to prevent disturbing the top of the column.

5. *Separation of acids:* When the surface of the chloroform is 2-3 mm. above the top of the sample section, 100 ml. of 0.75% butanol in chloroform is added. Then, 100 ml. of 5% butanol in chloroform is added. The first 150 ml. of solvent empties from the siphon into a 250-ml. Erlenmeyer flask and contains the higher fatty acids; the next 10 ml. of solvent is collected in a 25-ml. graduate cylinder and is free from acid. The remainder of solvent is collected in the siphon and contains the butyric acid.
6. *Titration* is made with 0.01 *N* base in absolute alcohol, using phenol red as an indicator. To avoid a false end point, 1 ml. of neutral absolute ethanol is added. Correction is made for the titration of the reagent blank, in which the sample is replaced with 2.0 ml. of water.
7. *Calculation* for butyric acid is made as follows:

$$\frac{\text{titration value for butyric acid}}{\text{Sum of the collected titration values for butyric acid and higher acids.}} \times 100 = \text{molar per cent of butyric acid.}$$

$$\text{Example: } \frac{3}{3+27} \times 100 = 10.0$$

The outlined procedure results in considerable saving of time over previous methods, permitting one analysis in 1 to 1½ hours, or four simultaneously in 2 hours. The use of the buffered column also enables the use of more rapid flow rate and additional time saving. During 200 analyses, 10 ml. of solvent collected just after the first 150 ml. gave blank titration values, indicating that complete separation is achieved and that an internal indicator is not necessary. Smaller columns can be used satisfactorily to save materials, and variations in flow rates up to 400 ml. per hour do not alter the results.

RESULTS

Development of method. To determine the reliability of the saponification procedure, two experiments were conducted. First, analyses for fatty acids in "saponified" fat-free mix were made to determine if the procedure would produce fatty acids by heat degradation of nonfat components. Second, saponification was carried out on cream and butter directly and on the fat extracted from the same products to demonstrate whether or not the presence of nonfat components

TABLE 1

Effect of the saponification procedure on the molar concentration of butyric acid in fat

Description of sample	Butyric acid
	(M%)
Fat free ice cream mix	0.0
Fat extracted from cream #1	10.6
Cream #1	10.4
Fat extracted from butter #1	10.5
Butter #1	10.5

would interfere with complete saponification of the fat and whether separation of the fat prior to saponification would be necessary. The representative data in Table 1 reveal that the saponification as conducted produced no fatty acids from nonfat components and also that the presence of milk components other than fat did not interfere with the efficiency of fat hydrolysis and recovery of butyric acid.

In a further study of the reliability of the procedure, recovery trials were made for both butyric acid and a mixture of higher acids. In order to determine if loss of fatty acids occurred during saponification, the acids were added both before and after this treatment. The data in Table 2 reveal that quantitative recoveries were obtained in all of the trials.

TABLE 2

Recovery of various acids added to ice cream mix.

Acid added	*N*/100 acid added to 0.6 ml. ice cream mix	No. trials	Recovery	
			Range	Average
	(ml.)		*(%)*	*(%)*
Butyric (before sapon.)	2.0	2	96.3-99.1	97.9
Butyric (after sapon.)	2.0	5	96.4-104.0	98.2
Mixed higher acids (before sapon.) [a]	7.0	2	94.7-101.2	97.9
Mixed higher acids (after sapon.)	7.0	4	98.2-103.1	101.3

[a] Mixed acids as follows: oleic acid 3.0 ml.; palmitic acid 1.0 ml.; stearic acid 1.0 ml.; laurie acid 1.0 ml.; and caproic acid 1.0 ml. *N*/100 acid.

TABLE 3

Measurement of butyric acid on replicate samples

Sample description	*Butyric acid (M%)*					
	1	2	3	4	5	Av.
Vanilla ice cream #12	9.8	10.0	9.9	9.9	10.1	9.9
Chocolate ice cream #2	8.3	8.5	8.3	8.1	8.4	8.3
Vanilla ice cream #10	10.3	10.2	10.3	10.0	—	10.2

To establish the reproducibility of the procedure, replicate analyses of three different samples of ice cream were made. Results, presented in Table 3, reveal an average mean variation of 3.0%. In another trial, in which two operators independently analyzed ten ice creams, variation never exceeded 0.4 *M*% of butyric acid, with an average variation of 0.2 *M*% or about ± 2.0% error.

Application to commercial ice cream. To determine the normal butyric acid content of commercial vanilla ice cream, a number of samples obtained from various parts of Ohio were analyzed. The results for 22 samples are shown in Table 4. They reveal a rather narrow range of butyric acid content, ranging from 9.5 to 10.7 *M*% butyric acid. Sixty-five per cent of the samples were between 10.0 and 10.2%, and 86% of the values were between 9.7 and 10.2%. These values agree with those obtained by Keeney *(6)* for ice cream samples obtained along the eastern seaboard.

TABLE 4

Butyric acid content of the fat in commercial vanilla ice cream

Sample	Butyric acid	Sample	Butyric acid
	(M%)		*(M%)*
1	10.0	12	9.9
2	9.5	13	10.1
3	10.1	14	10.0
4	10.1	15	10.0
5	10.05	16	9.8
6	10.1	17	9.6
7	10.0	18	10.0
8	10.7	19	10.6
9	10.0	20	9.7
10	10.2	21	9.9
11	10.1	22	9.7

A problem exists in the detection of substitute fat in chocolate ice cream, since the flavoring component provides a variable amount of vegetable fat, depending upon the composition and amount of chocolate products used. Therefore, trials were conducted to determine the normal butyric acid content of chocolate ice cream. Eight commercial samples were analyzed, and the results are presented in Table 5. These data reveal that the butyric acid content is much more variable in chocolate than in vanilla ice creams, ranging from 7.3 to 9.1 *M%*.

On the basis of the butyric acid values established for vanilla ice cream, the lowest values obtained for the chocolate ice creams represent a maximum substitution of about 25% fat, whereas the highest value obtained was for a chocolate ice cream containing 16% total fat. These results indicate that foreign fat substitution in chocolate ice cream in excess of that contributed by the flavoring may be detected, provided that the amount of chocolate and the fat content of the chocolate product are known. This emphasizes the need for adequate labeling of chocolate ice cream.

Determination of fat substitution. An investigation was made to ascertain the sensitivity of the method in determining the level of substitute fat which could be detected. (Study was also made to determine the quantitative value of the method. Both of these are of value in a method for measuring fat substitution.)

Several different types of fats of both vegetable and animal origin were blended with samples of known butyric acid values. Substitution of fat was made at 10, 20, 50, and 100% levels. Calculation of the amount of fat substituted was made from the butyric acid values, using Keeney's formula for calculation *(6)*. The butyric acid content of the fat and the calculated per cent substitution are

TABLE 5

Butyric acid content of the fat in commercial chocolate ice cream

Sample	Butyric acid	Sample	Butyric acid
	(M%)		*(M%)*
1	9.1	5	7.7
2	8.3	6	8.1
3	7.6	7	7.7
4	7.3	8	7.6

TABLE 6

Butyric acid content of fat in samples of known fat substitution

Dairy product	Fat substituted	Amount of substitute	Butyric acid	Calculated fat substitution from butyric acid value [a]	
				Formula 1	Formula 2
		(%)	*(M%)*	*(%)*	*(%)*
Butter	Margarine	0	10.7	——	——
		10	9.5	10.5	9.9
		50	6.3	56	39.2
		100	0.5	100	96.8
Ice cream #11	Hydrogenated coconut oil	0	10.1	——	——
		10	8.7	19.4	11.4
		20	7.9	24.5	20.0
		50	4.8	55.9	50.0
		100	0	——	100.0
Ice cream #20	Hydrogenated lard	0	9.7		
		10	8.3	15.0	13.0
		20	7.4	25.0	21.5
Ice cream #22	Unknown mixed hydrogenated fat	0	9.7	——	——
		10	8.9	11.0	9.5
		20	7.5	23.2	20.8
		50	5.5	52.1	40.4
		100	0.0	100.0	——

[a] A correction must be made for differences in the fatty acid composition of milk fat and substitute fat in order to estimate the amount of substitution. Calculations are based on Keeney's formula, except for fat containing coconut oil.

1. Keeney formula: (S = % substitution)

$$S = 100 - \frac{2000\ (\%\ \text{butyric in sample})}{23(\%\ \text{butyric in milkfat}) - 3(\%\ \text{butyric in sample})}$$

2. Corrected formula for coconut oil: (S = % substitution)

$$S = 100 - \frac{2620\ (\%\ \text{butyric in sample})}{23(\%\ \text{butyric in milkfat}) + 3(\%\ \text{butyric in sample})}$$

shown in Table 6 for one butter and three ice cream samples. Addition of 10% substitute fat resulted in a marked and measurable reduction of butyric acid in all cases. Even when using butter with the highest measured butyric acid content (10.7%), the addition of 10% margarine fat resulted in a butyric acid value lower than any values reported for a normal milkfat product. Substitution of over 10% fat further reduced the butyric acid concentration, approximately proportionate to the per cent substitution.

Calculation of the per cent substitution is possible only when the type of substitute fat and the butyric acid content of the milkfat are known. The data in Table 6 emphasize that the fatty acid composition of the substitute fat is important. Fats containing a larger amount of lower fatty acids, such as coconut oil, result in a greater reduction in butyric acid values than do other types of fat. This is important to consider, since coconut fat is one of the most difficult to detect in mixtures of butterfat in the Reichert-Meissl determination and also is frequently used as a substitute.

Therefore, to apply the butyric acid method accurately, some correction must

be made for differences in the fatty acid composition of butterfat and the substitute fat. Although one formula will suffice for most substitute fats, as indicated by Keeney *(6)*, the formula is not valid for coconut fat.

Since, as shown in Table 6, neither formula will apply to all samples, the quantitative degree of substitution cannot be determined on unknown samples. This is especially true at low levels of substitution.

SUMMARY

A method is presented for the rapid direct chromatographic determination of the molar concentration of butyric acid in the fat of dairy products which may be utilized as a means of detecting fat substitution in dairy products.

This method has the advantage of being readily adaptable to rapid routine analyses since it eliminates time-consuming fat extraction, simplifies the chromatographic separation, and provides a reproducible accurate method for measuring butyric acid in fat.

The butyric acid content of 26 commercial vanilla ice creams varied between 9.5 and 10.7%. Sixty per cent of the butyric acid values were above 10.0%, and 95% of the values were 9.6%, or higher. Chocolate ice cream had a butyric acid content ranging from 7.3 to 9.1%. Because of the wide variation in the fat level and the amount of chocolate used, no conclusion can be made as to the expected average normal value for chocolate ice cream.

Substitution of fat in ice cream may be detected readily at the 10% level for both vegetable and animal fats. However, the amount of fat substituted may be estimated only semi-quantitatively. The error involved in estimating the per cent substitution was as high as 50% but averaged 14%. When the source of substitute fat or the butyric acid content of the milk fat is unknown, the amount of substitution cannot be determined.

REFERENCES

(1) Bird, E. W., Patel, I. J., and Handwerk, R. L. Detection of Vegetable Fat in Milkfat by the Tocopherol Determination. *J. Dairy Sci.*, **34**: 484. (abs.) 1951.

(2) Bhalerao, V. R., and Kummerow, F. A. A New Method for the Detection of Foreign Fats in Dairy Products. *J. Dairy Sci.*, **36**: 567. (abs.) 1953.

(3) Chilson, W. H., Sommer, H. H., and Riel, Rene. The Detection of Foreign Fats in Dairy Products. *J. Dairy Sci.*, **36**: 566. (abs.) 1953.

(4) Harper, W. J. Direct Chromatographic Determination of Acetic, Propionic and Butyric Acids in Cheese. *J. Dairy Sci.*, **36**: 808. 1953.

(5) Irmiter, T. F. Unpublished Ph.D. dissertation. The Ohio State University. 1951.

(6) Keeney, Mark. Chromatographic Determination of Butyric Acid and the Detection of Milkfat Adulteration. Maryland Agr. Expt. Sta., *Misc. Pub.* 153. January, 1953.

(7) Keeney, Mark, A Presumptive Crystallization Test for Milkfat Adulteration. *Ice Cream Trade Journal*, **49** (6): 30. 1953.

(8) Lawrence, C. E. A Quick Test for Foreign Fats in Frozen Desserts. *Southern Dairy Products J.*, **52**: (12) 124. 1952.

(9) Roger, T. A. *Fundamentals of Dairy Science.* Chemical Catalog Company, Inc. p.77. 1928.

PARENTERAL ADMINISTRATION OF AUREOMYCIN TO YOUNG CALVES WITH A NOTE ON MODE OF ACTION[1]

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Several antibiotics have proved to be of value in the nutrition and well-being of chicks, turkeys, pigs, and other nonruminants. In ruminants, only aureomycin has been definitely established as being beneficial to young calves, resulting in increased growth, reduced incidence of scours, improved physical appearance and better feed efficiency up to 16 weeks of age *(2, 17, 20, 22, 29, 30, 31)*. Recent work indicates that terramycin is of some benefit to young calves *(18, 23, 34)*. However, the mode of action of antibiotics in the young ruminant is still unknown.

A few hypotheses have been advanced that the increased growth response from aureomycin supplementation is due to changes in the character of the rumen microflora. Several research workers have made an attempt to investigate the mechanism of antibiotic action by studying the effect of antibiotics in the rumen and also on the intestinal flora. The somewhat conflicting results probably can be explained by the different ages, rations, and experimental conditions employed in the various trials. In reporting unfavorable effects of feeding antibiotics to ruminants, Colby *et al. (7)* in 1950 suggested that rumen inhibition occurred when antibiotics (aureomycin, penicillin, and streptomycin) were fed to sheep, and Bell *et al. (5)* suggested that the cellulolytic organisms were affected, since a 50% decrease in digestibility of crude fiber was found in experiments with aureomycin-fed steers.

In studies with young calves responding to the growth-stimulating effect of aureomycin, several investigators *(3, 21, 31)* found no difference in the number or types of bacteria present in the rumen flora of aureomycin-fed and of control animals. Bartley *et al. (3)*, however, mentioned that preliminary cultural studies indicated some difference. Neumann *et al. (26)* suggested that the normal rumen flora was disturbed in aureomycin-fed yearlings, since the types of organisms present were less diverse. Using an artificial rumen, Kesler and Knodt *(18)* reported that rumen samples of terramycin-fed calves decreased cellulose digestion by 24%. Chance *et al. (6)*, in studies with rumen-fistulated steers, reported that aureomycin (0.5 g.) increased the number of bacteria and that higher levels

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of aureomycin inhibited some of the cellulose-digesting bacteria in the rumen. Wasserman *et al. (35)*, using the artificial rumen, found a stimulatory cellulolytic activity with low concentrations of penicillin and streptomycin and all concentrations of neomycin, whereas higher concentrations of streptomycin and chloromycetin inhibited cellulose digestion.

Bacteriological studies of the fecal contents of antibiotic-fed and of control calves have shown no consistent differences *(30, 34)*. Rusoff *et al. (30)* have recently concluded that the growth-stimulating effect of aureomycin in calves apparently was not due to selective bacterial inhibition of the common bacterial groups of the intestine, namely, coliforms, enterococci, and *Clostridium perfringens*.

In a preliminary study, Rusoff *et al. (32)* reported on the growth-promoting effect of parenteral administration of aureomycin to young calves. The growth response obtained by this method of administration could not accrue from its action in the rumen, since studies *(16, 19)* with other species (pig, dog, and man) have suggested that injected antibiotics enter the intestines via the bile, thus by-passing the rumen. Therefore, a study of the effect of intramuscular injections of aureomycin and oral-administered aureomycin on the young calf was made. Also, rumen flora and rumen environment studies, including B-vitamin content; pathways of excretion of aureomycin; and carcass, meat, and bone studies are presented in order to interpret the mode of action of antibiotics in calves.

EXPERIMENTAL

Growth studies. Three comparable groups of eight male Jersey calves each were used. The animals received colostrum for the first 3 days after birth and at 4 days of age were placed in individual pens with wood shavings. Each group received a 19% digestible plant protein calf starter. The starter consisted of cottonseed meal, 35.0%; soybean oil meal, 5.5%; yellow corn meal, 31.5%; crimped oats, 15.0%; wheat bran, 10.0%; steamed bonemeal, 2.0%; salt, 1.0%; and Quadrex 10 (Type IV),[3] 0.2%. Group I served as a control, Group II received weekly intramuscular (semi-tendinosus of hind leg) injections of 400 mg. aureomycin as an $AlCl_3$ complex in sesame oil, and Group III received orally a tablet containing 50 mg. aureomycin per day for the first 30-60 days and Aurofac A supplement (1.8 g. aureomycin per pound) at a 1% level in the calf starter. The tablets were fed in the milk until the animals were consuming sufficient starter to approximate the level of aureomycin in the tablet, i.e., 50 mg. Thus, the injected aureomycin calves (Group II) and the oral-fed aureomycin calves (Group III) were obtaining approximately the same quantity of aureomycin per week.

All calves were fed milk from a nipple pail at the rate of 1 lb. per 10 lb. of body weight to 30 days of age. The calf starters were fed ad libitum from the eighth day of age until a maximum of 5 lb. was being consumed daily. A medium-

[3] Micratized vitamin supplement containing 10,000 A and 1,250 D_2 per gram, supplied through the courtesy of M. Hochberg, NOPO Chemical Co., Harrison, N. J.

grade Alyce clover hay also was fed ad libitum on the eighth day. All calves were weighed at weekly intervals through 16 weeks of age. Measurements of height at withers were made at 4 days and at 16 weeks of age. Daily observations of physical appearance and consistency of the feces were made for each calf. Statistical analysis of the data was made according to the method of Snedecor *(33)*.

Rumen studies. Twice during the experiment rumen samples from most of the calves (13-15 weeks of age) were taken by stomach tubes, as described by Gall *et al. (8)*. The samples were studied for pH, Eh, degree of fiber digestion in the miniature artificial rumen, and types of rumen organisms by Gram stain of the original sample and by an anaerobic cultural series. The pH and Eh determinations were made immediately on the rumen fluid with the Beckman meter, using the glass and platinum electrodes, respectively. The miniature artificial rumen technique used was that presented by Huhtanen and Gall *(12)*, and the anaerobic cultural technique was that described by Huhtanen *et al. (15)* and modified by Huhtanen and Gall *(13)*. The organisms isolated are described in three publications *(9, 13, 14)*. The B-vitamin determinations were done on the pooled rumen fluid from each group of calves by slight modifications of commonly employed microbiological assay techniques *(4)*.

Aureomycin assay. To determine pathways of excretion of aureomycin, samples of blood and feces were obtained from all calves at 15-16 weeks of age and pooled according to group. Pooled samples of bile and urine were obtained from the two calves of each group which were slaughtered. These samples were obtained during the fourth day after the animals in Group II were injected. Aureomycin assays were made of these samples by a modification of the dip technique of the pad-plate method of Grady and Williams *(11)*. Duplicate determinations were made from each sample and standard curves were prepared in the control of each sample.

Carcass studies. Two animals of each group, which were placed on experiment at the same time, were slaughtered at the completion of the experiment. Carcass studies, meat yields, and meat quality studies were made on each carcass according to procedures recommended by the Bureau of Animal Industry, U.S.D.A. *(1, 25)*.

RESULTS AND DISCUSSION

Growth studies. The data concerning the average daily gains, feed efficiency, and increase in height at withers are presented in Table 1. Aureomycin, either oral-fed or injected, stimulated the growth of calves. The injected calves (Group II) showed an increase in weight of 30% over the control calves, and the oral-fed calves (Group III) showed an increase of 20%. These differences were statistically significant at $P < 0.05$. The "t" test showed that there were no significant differences at $P < 0.05$ between the weight gains of the injected group of calves and the oral-fed group.

In regard to feed efficiency, the calves which received aureomycin, particularly those which were injected, started grain consumption at an earlier age than did

TABLE 1

Average daily gains, increase in height of withers, and feed efficiency of dairy calves at 16 weeks of age

Group	Birth wt.	Av. daily gain[a]	Starter per lb. gain	Height at withers	
				Initial ht.	Increase in ht.[a]
	(lb.)	*(lb.)*	*(lb.)*	*(in.)*	*(in.)*
I Control	54.4	0.78	3.13	25.59	5.99
II Injected Aureomycin	55.3	1.07	2.67	25.54	7.59
III Oral Aureomycin	55.3	0.94	2.79	25.42	6.44

[a] $P < 0.05$

the control calves. Although the antibiotic groups of calves showed a better feed efficiency than did the control group, the differences were not statistically significant. Hay consumption was approximately the same for all groups. The aureomycin groups of calves, particularly those fed orally, consumed slightly more water than did the control group.

Both aureomycin groups of calves showed a greater increase in height at withers than the control group. These differences were statistically significant at $P < 0.05$. Although the aureomycin-injected group showed the greatest increase in height, the difference between the injected and oral-fed aureomycin groups was not significant at $P < 0.05$. Since this measurement reflects the growth of skeletal tissue, it suggests a stimulation of skeletal growth.

Only a few cases of scours occurred, and these were mainly among the calves of the control group.

When the animals were 9-10 weeks of age, the hair coat of the aureomycin-injected calves was markedly superior to that of the calves in the two other groups (Figure 1). At 12-16 weeks of age this condition was observed in all the calves in the oral aureomycin-fed group. Only three of the animals in the control group showed this smooth hair coat at the completion of the experiment (16 weeks).

Rumen studies. The data in Table 2 show that the rumen environment was similar in all groups of calves. The degree of fiber digestion was fair in most

FIG. 1. At 3 months of age—oral aureomycin-fed calf on left and aureomycin-injected calf on right.

TABLE 2

Data from rumen samples

Group	Calf No.	pH	Eh	Degree of fiber digestion in artificial rumen [c]		Gram stain [d]	Flora	
							Predominating organisms	Evaluation [e]
		1 [a]	2 [b] (*mv.*)	1	2	2	2	2
Control	22	6.70	−150	fair	fair	poor	*6TBR*, L, PR	poor
	26	6.74	−100	fair	neg.	good	*T*, *6TBR*, SCC, PR	poor
	30	6.80	−100	fair	neg.	poor	*6TBR*, *L*, *HD*, PR	good
	31	6.90	−150	not done	fair	poor	*H*, *6TBR*, PR, LCC	good
	33	6.20	−200	not done	neg.	good	*6TBR*	poor
	36	6.40	−100	poor	poor	poor	*6TBR*, PR, C8, H	fair
	40	6.50	−150	fair	poor	poor	*H*, *6TBR*, PR, LCC	good
	Av.	6.6	−136					
Injected	21	6.42	−150	poor	——	good	*T*, *H*, 6TBR	good
	24	6.95	−200	fair	fair	good	*T*, *PR*, *L*, C8, 6TBR, HD	fair
	27	6.56	−150	fair	fair	good	*H*, *T*, 6TBR, HD, C8	good
	28	7.17	−225	fair	fair	good	*H*, *HD*, L	very good
	34	5.80	−150	poor	poor	poor	*H*, *T*, *PR*, C8, 6TBR, LCC	good
	35	6.10	−160	fair	poor	good	*6TBR*, HD, L, T	fair
	Av.	6.5	−172					
Oral	23	7.10	−120	neg.	neg.	poor	*6TBR*, *HD*, *H*, *TRC*, *L*	very good
	25	7.00	−150	poor	poor	poor	*6TBR*	poor
	29	6.85	−130	poor	poor	poor	*H*, SCC, PR	good
	32	6.75	−150	neg.	neg.	good	*6TBR*	poor
	38	6.65	−150	neg.	fair	poor	*H*, *PR*, *6TBR*	good
	42	6.78	−180	poor	fair	poor	*6TBR*, H, HD	fair
	Av.	6.9	−147					

[a] 1st sampling.

[b] 2nd sampling two weeks later.

[c] Good, 40% or above; fair, 20-39%; poor, 10-19%; negative, 0-9%.

[d] Evaluation of flora is based on parasitism of fibers and morphological types of organisms observed.

[e] Evaluation of flora is based on ability of rumen organisms isolated to carry on important rumen functions, especially fiber digestion.

of the control and aureomycin-injected animals on both tests but was somewhat depressed in the calves in the oral-fed group, particularly at the time of the first testing.

The types of rumen organisms, based on visual observations and anaerobic cultures and isolation studies, were similar in all groups. Most animals had a predominating flora of the grain-digesting types of organisms with limited roughage-digesting types. The rumen fluids of some calves had more roughage-digesting types than others and they were evaluated as carrying better flora.

The B-vitamin content of the rumen fluid was similar for all groups of animals (Table 3). Thus, there were no clear-cut differences in the rumen flora and environment between the three groups of animals. This confirms previous findings that ruminants fed liberal amounts of grain, as in this experiment, do not seem to be markedly affected with respect to rumen flora and environment by moderate intake of antibiotics.

Aureomycin assay. The pathways of excretion of injected and oral-administered aureomycin are shown in Table 4. Examination of the data shows that injected aureomycin is excreted mainly in the urine, whereas oral-fed aureomycin

TABLE 3

B vitamin assay of rumen contents (13-15 weeks)

Vitamin	Control	Injected	Oral
	(γ/ml)	*(γ/ml)*	*(γ/ml)*
B_{12}	0.0087	0.0062	0.0064
Pantothenic Acid	1.29	0.92	0.95
Riboflavin	1.51	1.07	1.04
Niacin	3.1	3.0	2.0
Biotin	0.02	0.014	0.014
Pyridoxine	0.45	0.44	0.33
Thiamine	0.10	0.066	0.21
Folic Acid	0.026	0.04	0.02

TABLE 4

Aureomycin content of body fluids and feces

Group	Blood	Bile	Urine	Feces
	(γ/ml)	*(γ/ml)*	*(γ/ml)*	*(γ/ml)*
Control	neg.	neg.	neg.	neg.
Injected	neg.	0.077	0.245	0.10
Oral	neg.	0.049	0.05	0.63

is excreted mainly in the feces. The concentration of aureomycin in the bile of the different groups of calves indicates that injected aureomycin is accumulated by the liver with subsequent excretion via the bile, which is the probable route by which injected aureomycin reaches the intestinal tract and feces. Jacob *et al.* *(16)* surmised that in man and in dogs injected aureomycin probably was excreted via the bile, and Larson and Carpenter *(19)* have suggested a similar pathway in pigs. The fact that small amounts of aureomycin are present in the bile and urine of the oral-fed calves indicates that some of the aureomycin is being ab-

sorbed and excreted by these pathways. No aureomycin was detected in the blood of the injected or oral-fed calves.

Although an aureomycin determination was not made on the rumen contents of the injected calves, it seems probable that no injected aureomycin, or very little, would be present. The data on the aureomycin concentrations found in the bile and feces of these animals appear to support this hypothesis. If injected aureomycin by-passes the rumen and, as the data indicate, enters the intestines via the bile, then the stimulatory effect of the antibiotic in young calves is not specifically through its action on the rumen microflora. Our results, which show no significant differences in the rumen microflora and rumen environment of the three groups of calves in this investigation, as well as our previous report *(31)* and those of others *(3, 21)*, appear to verify this contention. It is possible that the intestinal microflora is affected by the antibiotic. However, the small amount of aureomycin present in the intestines of the injected calves, as seen in the concentrations found in the bile and feces, and the results of our recent report *(30)*, in which oral-fed aureomycin did not affect the common bacterial groups of the intestines, discount this hypothesis. From our results, it is suggested that a stimulation of tissue activity or some biochemical mechanism appears to be involved.

Some support for this hypothesis is presented by a few investigators who have pointed out that antibiotics may influence mineral absorption and utilization. Migicovsky *et al. (24)* concluded that penicillin enhanced the absorption of calcium in baby chicks. Ross and Yacowitz *(28)* reported that penicillin appeared to decrease the vitamin D requirements for normal bone calcification in chicks, and Pepper *et al. (27)* concluded that aureomycin lowered the amount of manganese required for growth and perosis prevention in chicks.

Carcass studies. Weights and measurements of the carcasses and bones of the representative calves in each group are presented in Table 5. It can be seen that the aureomycin groups of calves, especially the injected animals, showed heavier weight and larger size of the carcass and bone than the control calves. In the physical separation of the 12th rib (Table 6), the bone and meat of the rib from the calves receiving aureomycin were heavier than those of the control calves. The chemical composition of the meat of the 12th rib from animals of the different groups (Table 7) shows a higher percentage of fat in the meat of the injected calves. The percentages of water, protein, and ash are approximately the same for all groups.

TABLE 5

Carcass weights and measurements, and bone measurements

Group	Carcass wt.	Length				
		Body	Foreshank	Hindshank	Humerus	Femur
	(lb.)	*(cm.)*	*(cm.)*	*(cm.)*	*(cm.)*	*(cm.)*
Control	74.8	727	187.5	284.5	179.0	228
Injected	110.5	832	222.0	306.0	191.5	249
Oral	95.8	795	212.0	300.0	185.0	238

TABLE 6
Physical separation of 12th rib

Group	Bone	Meat
	(g.)	*(g.)*
Control	25.5	84.0
Injected	55.5	123.5
Oral	33.0	108.0

TABLE 7
Chemical analyses of meat (12th rib)

Group	Moisture	Protein	Ash	Fat
	(%)	*(%)*	*(%)*	*(%)*
Control	68.95	29.1	1.53	2.82
Injected	65.87	27.6	1.39	7.28
Oral	67.82	28.4	1.37	3.62

These results of the carcass studies indicate that general growth was stimulated, and it is possible that the pituitary growth hormone is involved. The data on increase of height at withers and on bone size and weight, however, suggest that bone metabolism might be the area in which aureomycin exerts its effect.

SUMMARY

Parenteral administration (intramuscular injection) of 400 mg. of aureomycin to young calves once weekly increased growth by 30% over controls at 16 weeks of age. Aureomycin administered orally at a level of 50-90 mg. daily stimulated growth by 20%. The antibiotic also caused a significant increase in height at withers.

Rumen studies of control calves, aureomycin-injected calves, and those fed aureomycin orally showed no significant differences in rumen environment (pH, Eh), degree of fiber digestion in the artificial rumen, rumen flora, and B-vitamin content (B_{12}, pantothenic acid, riboflavin, niacin, biotin, pyridoxine, thiamine, and folic acid) of rumen fluid.

Pathways of excretion of injected aureomycin are mainly through the urine and bile, probably by-passing the rumen, whereas oral-fed aureomycin is excreted mainly in the feces.

In carcass studies the aureomycin-injected calves and those fed aureomycin orally showed larger skeletal size and more meat than did the controls.

The mode of action of aureomycin in stimulating growth in the young calf is not due to its action in the rumen. It is suggested that bone metabolism may be the area in which aureomycin exerts its effect.

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REFERENCES

(1) Animal Industry Division, B.A.I., U.S.D.A. National Cooperative Project. A Study of the Factors Which Influence the Quality and Palatability of Meat. *Mimeo.* Rev. ed. 1927.

(2) Bartley, E. E., Fountaine, F. C., Atkeson, F. W., and Fryer, H. C. Antibiotics in Dairy Cattle Nutrition. I. The Effect of an Aureomycin Product (Aurofac) on the Growth and Well-Being of Young Dairy Calves. *J. Dairy Sci.*, **36**: 103. 1953.

(3) Bartley, E. E., Wheatcroft, K. L., Claydon, T. J., Fountaine, F. C., and Parrish, D. B. Effects of Feeding Aureomycin to Dairy Calves. (Abs.) *J. Animal Sci.*, **10**: 1036. 1951.

(4) Barton-Wright, E. C. *The Microbiological Assay of the Vitamin B Complex and Amino Acids.* Sir Isaac Pitman and Sons, Ltd. London. 1952.

(5) Bell, M. C., Whitehair, C. K., and Gallup, W. D. The Effect of Aureomycin on Digestion in Steers. (Abs.) *J. Animal Sci.*, **9**: 647. 1950.

(6) Chance, C. M., Huffman, C. F., and Duncan, C. W. Antibiotics in Rumen Digestion and Synthesis. I. The Passage of Nutrients from the Rumen During Specific Intervals After Feeding. *J. Dairy Sci.*, **36**: 253. 1953.

(7) Colby, R. W., Rau, F. A., and Miller, J. C. The Effects of Various Antibiotics on Fattening Lambs. (Abs.) *J. Animal Sci.*, **9**: 652. 1950.

(8) Gall, L. S., Burroughs, W., Gerlaugh, P., and Edgington, B. H. Special Methods for Rumen Bacterial Studies in the Field. *J. Animal Sci.*, **8**: 433. 1949.

(9) Gall, L. S., and Huhtanen, C. N. Criteria for Judging a True Rumen Organism and a Description of Five Rumen Bacteria. *J. Dairy Sci.*, **34**: 358. 1951.

(10) Gall, L. S., Huhtanen, C. N., Saunders, R., and Schmidt, W. Comparison of Rumen Flora and Environment in Roughage *vs.* Grain-Fed Animals. *J. Dairy Sci.*, **36**: 587. 1953.

(11) Grady, J. E., and Williams, W. L. Determination of Aureomycin in Feeds by the Pad-Plate Method. *Antibiotics & Chemotherapy*, **3**: 158. 1953.

(12) Huhtanen, C. N., and Gall, L. S. The Miniature Artificial Rumen and Its Uses. (Abs.) *J. Animal Sci.*, **11**: 766. 1952.

(13) Huhtanen, C. N., and Gall, L. S. Rumen Organisms. I. Curved Rods and a Related Rod Type. *J. Bacteriol.*, **65**: 548. 1953.

(14) Huhtanen, C. N., and Gall, L. S. Rumen Organisms. II. Two Lactate Utilizers and Six Miscellaneous Types. *J. Bacteriol.*, **65**: 554. 1953.

(15) Huhtanen, C. N., Rogers, M. R., and Gall, L. S. Improved Techniques for Isolating and Purifying Rumen Organisms. *J. Bacteriol.*, **64**: 17. 1952.

(16) Jacob, S., Schweinburg, F. B., and Rutenburg, A. M. Effect of Intravenous Aureomycin on the Intestinal Flora of Dog and Man. *Proc. Soc. Exptl. Biol. Med.*, **78**: 121. 1951.

(17) Jacobson, N. L., Kaffetzakis, J. G., and Homeyer, P. G. The Effect of Aureomycin Feeding on Changes in Weight and in Body Measurements of Dairy Calves. *J. Dairy Sci.*, **35**: 1094. 1952.

(18) Kesler, E. M., and Knodt, C. B. Effect of Terramycin on Growth and Rumen Function in Holstein Calves. (Abs.) *J. Animal Sci.*, **11**: 768. 1952.

(19) Larson, W. L., and Carpenter, L. E. The Fecal Excretion of Aureomycin by the Pig. *Arch. Biochem. and Biophys.*, **36**: 239. 1952.

(20) Loosli, J. K., and Wallace, H. D. Influence of APF and Aureomycin in the Growth of Dairy Calves. *Proc. Soc. Exptl. Biol. Med.*, **75**: 531. 1950.

(21) Loosli, J. K., Wasserman, R. H., and Gall, L. S. Antibiotic Studies with Dairy Calves. (Abs.) *J. Dairy Sci.*, **34**: 500. 1951.

(22) MacKay, A. M., Riddell, W. H., and Fitzsimmons, R. Feed Supplement Containing Aureomycin and Vitamin B_{12} for Dairy Calves. *J. Animal Sci.*, **11**: 341. 1952.

(23) MacKay, A. M., Riddell, W. H., and Fitzsimmons, R. Terramycin Supplement for Dairy Calves. *J. Animal Sci.*, **12**: 19. 1953.

(24) Migicovsky, B. B., Nielson, A. M., Gluck, M., and Burgess, R. Penicillin and Calcium Absorption. *Arch. Biochem. and Biophys.*, **34**: 479. 1951.

(25) National Livestock and Meat Board. Fourth Annual Reciprocal Meat Board, Chicago. *Rpt. of Proceedings.* 1951.

(26) Neumann, A. L., Snapp, R. R., and Gall, L. S. The Long Time Effect of Feeding Aureomycin to Fattening Beef Cattle, with Bacteriological Data. (Abs.) *J. Animal Sci.*, **10**: 1058. 1951.

(27) Pepper, W. F., Slinger, S. J., and Motzok, I. The Effect of Aureomycin on the Interrelationship Between Manganese and Salt in Chicks. (Abs.) *Poultry Sci.*, **30**: 926. 1951.

(28) Ross, E., and Yacowitz, H. The Effect of Penicillin on Vitamin D Requirements for Growth and Bone Calcification. (Abs.) *Poultry Sci.*, **31**: 933. 1952.

(29) Rusoff, L. L. Antibiotic Feed Supplement (Aureomycin) for Dairy Calves. *J. Dairy Sci.*, **34**: 652. 1951.

(30) Rusoff, L. L., Alford, J. A., and Hyde, C. E. Effect of Type of Protein on the Response of Young Dairy Calves to Aureomycin with Data on the Intestinal Microflora. *J. Dairy Sci.*, **36**: 45. 1953.

(31) Rusoff, L. L., Davis, A. V., and Alford, J. A. Growth-Promoting Effect of Aureomycin on Young Calves Weaned from Milk at an Early Age. *J. Nutrition*, **45**: 289. 1951.

(32) Rusoff, L. L., Fussell, J. M., Hyde, C. E., and Crown, R. M. Oral Supplementation Versus Intramuscular Injection of Aureomycin to Young Calves. (Abs.) *J. Dairy Sci.*, **36**: 593. 1953.

(33) Snedecor, G. W. *Statistical Methods.* Iowa State College Press, Ames. 1946.

(34) Voelker, H. H., and Cason, J. L. Antibiotics Studies with Dairy Calves. (Abs.) *J. Animal Sci.*, **10**: 1065. 1951.

(35) Wasserman, R. H., Duncan, C. W., Churchill, E. S., and Huffman, C. P. The Effect of Antibiotics on in Vitro Cellulose Digestion by Rumen Microorganisms. *J. Dairy Sci.*, **35**: 571. 1952.

APYRASE ACTIVITY OF THE MAMMARY GLAND[1, 2, 3]

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Manometric study of hexokinase activity of mammary gland homogenates *(3)* was complicated by high control values, e.g., release of carbon dioxide in the absence of hexose substrate. This carbon dioxide release was readily attributed to hydrolysis of adenosinetriphosphate (ATP).

The enzymatic hydrolysis of ATP has been studied extensively with preparations from muscle. When this hydrolytic activity was associated with the myosin fraction of the muscle proteins *(4, 8)* interest in the observation became widespread, and similar activity was subsequently found in a variety of other animal tissues. Enzymes capable of releasing inorganic phosphate from adenylpyrophosphates have been designated by the general term, apyrase, whereas an adenosinetriphosphate (ATPase) specifically removes only the terminal phosphate group from ATP.

Crude homogenates and water extracts of mammary gland tissue from the lactating rat were found to contain apyrase. Inasmuch as the reactions involving ATP may be of considerable importance in the synthesis of milk, this reaction has been studied in detail. The results of this investigation are the subject of this report.

EXPERIMENTAL

The hydrolysis of phosphate esters which produces new acid groups results in the release of carbon dioxide in the presence of bicarbonate. Manometric measure of this gas afforded an assay of hydrolytic activity. In some experiments the action was followed by measuring the release of inorganic phosphate (iP).

Variation was observed in the results of preliminary experiments which seemed to be related to the amount of residual milk in the gland. To control this factor the following procedure was adopted. Holtzman rats which had been lactating at least 15 days were separated from their litters for at least 4 hours. To effect removal of the milk, two litters were allowed to nurse one rat. After the rat was decapitated, the mammary glands were removed rapidly and frozen in powdered dry ice. The glands located in the abdominal-inguinal region were used most frequently because of the ease of removal and the absence of inter-

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[2] A portion of these data was taken from a thesis presented by E. M. Craine to the Graduate Faculty of the University of Illinois to fulfill partial requirements for the degree of Doctor of Philosophy.

[3] Supported in part by a grant from the United States Atomic Energy Commission [Contract No. AT(11-1)-67, Project No. 10].

lacing muscle tissue. The frozen tissue was powdered in a stainless steel cylinder *(9)* which was chilled by an acetone-dry ice bath. The powdered tissue was homogenized in a cold glass homogenizer with sufficient medium (water or 0.015 *M* phosphate buffer at pH 7.4) to make a 20% homogenate. After filtration through gauze, this fluid was used as the crude homogenate. These preparations maintained their activity at low temperatures; thus for some experiments they were stored in powdered dry ice.

Some of the crude homogenates were allowed to stand at 0° C. for 15 minutes and were then centrifuged at 0° C. in various gravitational fields as described in the results. The supernatant obtained was designated an extract. When a second extraction was made, the residue was suspended in a small amount of cold water; extracting fluid was then added, and the mixture was allowed to stand 15 minutes and then centrifuged at 0° C. In some cases the residue was suspended in cold water; this preparation was referred to as cellular debris. Protein was determined by a semi-micro Kjeldahl method.

For manometric experiments the following constituents (final concentration) were added to all flasks in order: 0.027 *M* potassium bicarbonate, 0.002 *M* potassium iodoacetate, 0.0015 *M* potassium phosphate (pH 7.4), 10 micromoles of ATP,[4] water to make a total volume of 2.0 ml. and 0.4 ml. of homogenate; 0.008 *M* magnesium sulfate and 0.05 *M* potassium fluoride were added in selected flasks. After the flasks were gassed with a mixture of 95% nitrogen–5% carbon dioxide, the ATP was added from a side arm to start the reaction. Incubations were performed at 38° C. Values obtained were corrected for retention of carbon dioxide. Portions of the incubation fluid were deproteinized with 10% trichloroacetic acid (TCA) and the iP content was determined in the clear supernatants by the method of Fiske and Subbarow *(5)*.

The experiments with the particulate fractions were conducted in small beakers of a shaker-incubator at 38° C. Five micromoles of ATP were incubated with mammary preparations in either 0.02 *M* acetate–cacodylic acid buffer (pH 7.1) or 0.027 *M* potassium bicarbonate (pH 8.2). Some beakers contained 0.008 *M* magnesium sulfate or calcium chloride. After equilibration, the ATP was added and the reaction was stopped at various intervals with strong TCA. Ten per cent TCA extracts were centrifuged and iP was determined on aliquots of the clear supernatants.

RESULTS

Magnesium effect. In Figure 1 the results of typical manometric experiments are presented. In the presence of added ATP, mammary gland homogenates released carbon dioxide rapidly from bicarbonate; this release of gas was linear for a period of 40 minutes. Where homogenates were prepared with water (Figure 1a) apyrase was stimulated by magnesium in the early phase of incubation, but the effect lessened with time. The result was similar in the presence

[4] The disodium salt obtained from Pabst Laboratories was neutralized with potassium hydroxide.

of fluoride but occurred to a lesser degree. In direct contrast to the observed magnesium stimulation with water homogenates, the addition of magnesium to homogenates prepared with phosphate buffer caused a depression of the carbon dioxide release (Figure 1b). This depression averaged 44% for 60-minute incubations.

Endogenous magnesium concentrations which were evidently due to the milk content of the tissue varied the stimulation considerably (Table 1). Greater

TABLE 1

Magnesium stimulation of apyrase activity [a]

Preparation	5 minute incubation		30 minute incubation	
	CO_2 released	Magnesium stimulation	CO_2 released	Magnesium stimulation
	(micromoles)	*(%)*	*(micromoles)*	*(%)*
Homogenate	4.3	79	14.1	6
Dialyzed homogenate	2.4	175	9.3	54
Extract [b]	3.7	56	10.2	10
Cellular debris	1.7	345	6.9	67

[a] ATP concentration: 10 micromoles per flask.
[b] Cellular debris separated by centrifugation at 2000 × G for 5 minutes.

effects occurred with dialyzed material or cellular debris where the concentration of ions had been decreased. The stimulation in 5 minutes was as much as 500 to 600% with cellular debris.

Fluoride inhibition. The strong inhibition of the enzyme by fluoride is evident from Figure 1. This inhibition occurred to the extent of about 70% in 60 minutes. The inhibition was the same under all conditions tested; both CO_2 anl iP released were affected to the same extent.

Inorganic phosphate–carbon dioxide ratios. If these actions had been due to the stepwise hydrolytic removal of phosphate groups from ATP, one micromole of carbon dioxide would have been released for every micromole of iP formed.

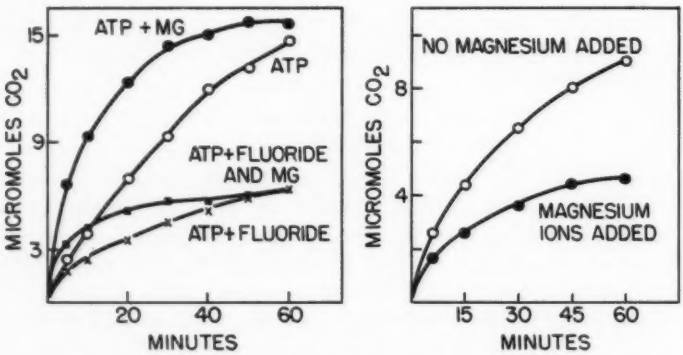

FIG. 1. The effect of magnesium and fluoride ions on the apyrase activity of mammary tissue homogenates: *(a)* water homogenate; *(b)* phosphate buffer homogenate.

TABLE 2

Molar ratios of iP formation to CO_2 release resulting from enzymatic hydrolysis of ATP

Preparation	No magnesium added	Magnesium added
30 minutes		
Homogenate	0.91 (3) [a]	1.05 (3)
Extract	1.07 (3)	1.12 (1)
60 minutes		
Homogenate		2.29 (4)
Extract		1.84 (2)
Dialyzed homogenate	1.23 (1)	1.28 (1)
Cellular debris	1.12 (3)	0.85 (3)

[a] Figures in parentheses indicate number of experiments.

This theoretical ratio of iP to carbon dioxide was observed in some of the manometric experiments (Table 2). Although there was considerable variation in these ratios iP/CO_2, most of the values obtained were approximately one or higher. The effects noted were independent of the presence of magnesium ions.

Fractionation. The above ratios indicated that several enzymes were producing the results observed. Attempts at separation were made on the basis of association with particulate matter of the tissue. A homogenate was prepared and extracted twice with water, and the remaining cellular debris was made into a homogenate. The original homogenate and the three derivatives were assayed for apyrase activity; the average data from several fractionations are presented (Table 3) as units per milligram of protein. A unit is equivalent to a micromole of iP released per 20 minutes of incubation.

The original apyrase activity of the crude homogenate which was measured without the addition of magnesium ions was recovered to the extent of 80% in the three preparations. The total activity measured in the presence of magnesium ions increased in the fractionation process. About half of the protein and 45% of the recovered apyrase activity were found in the extracts, which were not clear solutions and must have contained mitochondria. The remaining 55% was associated with the cellular debris of the homogenate which contained 40% of the original protein. The activity per milligram of protein was highest in the debris fraction.

TABLE 3

Fractionation of mammary apyrase on the basis of association with particulate material [a]

Fraction [b]	Protein extracted	No magnesium added		Magnesium added	
	(%)	*(Units per mg. protein)*	*(% of total activity)*	*(Units per mg. protein)*	*(% of total activity)*
Crude homogenate		0.77		0.99	
Extract 1	38	0.71	28	0.86	39
Extract 2	13	0.37	6	1.11	16
Extract 3	40	1.07	49	1.26	65
Percent recovered	91		83		120

[a] Incubations in acetate-cacodylic buffer. ATP concentration: 5 micromoles.
[b] Extractions prepared by centrifugation at 10,000 × G for 10 minutes.

TABLE 4

Ion influences on the enzymatic hydrolysis of ATP [a]

Preparation [b]	iP released from ATP in acetate-cacodylic buffer	Increase by		
		Mg	Ca	Bicarbonate buffer
	(micromoles)	*(%)*	*(%)*	*(%)*
Extract 1	5.4	11	0	74
Extract 2	1.4	150	135	226
Cellular debris	7.9	16	−5	27

[a] ATP concentration: 5 micromoles.
[b] Extractions prepared by centrifugation at 10,000 × G for 10 minutes.

The addition of magnesium stimulated activity in all three fractions (Table 4, where a unit is equivalent to a micromole of iP released per 40 minutes); the greatest increase was in Extract 2, where it averaged two and one-half fold. Calcium addition was ineffective except in Extract 2, although it was not as effective for stimulation as magnesium.

As shown in Table 4, the entire hydrolytic action was increased when bicarbonate (pH 8.2) was used in place of the acetate-cacodylic buffer (pH 7.1). This was particularly true in the water extract; Extract 2 averaged 226% more activity where bicarbonate replaced the acetate-cacodylic buffer. When the incubation was allowed to proceed 40 minutes in bicarbonate, as much as 12 micromoles of iP were released from 5 micromoles of ATP. This did not occur even at 60 minutes in the acetate-cacodylic incubations.

At low protein concentrations the release of iP by the water extract was a linear function for as long as 40 minutes (Figure 2a). In most of the experiments the linearity was evident for the first 20 minutes. In the ranges tested the activity was a linear function of the amount of preparation or protein used (Figure 2b); this held true at low enzyme concentrations in both the presence and absence of magnesium.

DISCUSSION

From the tissue of cow mammary glands, Borst *(2)* was able to isolate a crystalline material identified as adenylic acid. Barrenscheen and Alders *(1)*

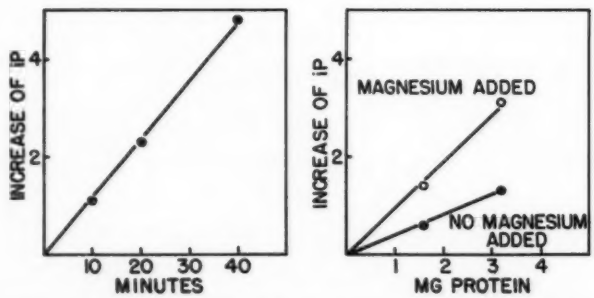

FIG. 2. Enzyme activity of water extract in the presence of 0.02 *M* acetate-cacodylic buffer: *(a)* time relation; *(b)* relation of protein concentration.

found in cow glands a 7-minute acid hydrolyzable phosphate fraction which they assumed to be primarily ATP. These results, together with data obtained in this laboratory, suggest that ATP is important to mammary gland metabolism.

Since two phosphates are removed from ATP during incubation at pH 7.1, the presence of a specific ATPase seems likely. The data do not explain the exact mechanism for the removal of the second phosphate group. It may be removed by the action of an ADPase or through the action of a myokinase *(7)*, which forms ATP and adenylic acid from ADP by phosphate transfer. Adenylic acid, the product remaining when two phosphates are split from ATP, is evidently not hydrolyzed in acetate-cacodylic buffer at pH 7.1. However, this third phosphate of ATP is hydrolyzed in bicarbonate; this may be due to the so-called "alkaline phosphatases" present in mammary tissue *(6)*. The actions occurring at pH 7 may be mediated by a single enzyme which is specific with respect to pyrophosphate bonds.

The water extractions indicate that there probably are several rather specific enzymes involved. Ability to remove two of the three phosphates of ATP is present both in water extracts and in the structural elements of the tissue. However, the two apyrases differ with respect to their activation by ions. The enzyme(s) of the extract is stimulated by both calcium and magnesium. This is more evident in the second water extraction; most of the soluble ions are removed by the first extraction, thus allowing the large activation by the added ions in the second extraction. Only small amounts of soluble magnesium and calcium would be present in the cellular debris. Thus the low order of stimulation from added ions in the latter may be due to the presence of a minor amount of the apyrase present primarily in the water extractions. The enzymes of the extract may be soluble or associated with mitochondria.

Magnesium has a diametric effect on apyrase in phosphate buffer and apyrase in water homogenates. It is known that during incubation of the water homogenates with ATP the iP content gradually increases so that the concentrations approach those in the phosphate buffer homogenates. The stimulation observed in the early phase of incubation seems to wane and may correspond to the continuous inhibition with phosphate preparations. The apparent inhibition seems to require magnesium and phosphate but the mechanism is not evident from these studies.

The wide variation of ratios of iP to carbon dioxide probably is due to the numerous reactions occurring in such a crude homogenate. This variation does not make the manometric method suitable for quantitative measure of ATPase or apyrase on crude mammary gland preparations. However, it has been useful for qualitative preliminary studies.

SUMMARY

An enzymatic hydrolysis of ATP by preparations of lactating rat mammary gland has been observed. The action was a linear function of time and protein concentration. Added magnesium ions depressed apyrase activity of homogenates made with phosphate buffer. In contrast, the addition of magnesium to water

homogenates produced a stimulation. Fluoride inhibited the apyrase action of either preparation.

About 45% of the apyrase was extracted with water. The enzymatic activity of this extract was stimulated with either calcium or magnesium ions. The remaining apyrase was associated with cellular debris of the mammary tissue. Variation in the stimulation with ions suggested the presence of multiple enzymes. The enzymes in both fractions removed at least two phosphates from ATP.

ACKNOWLEDGMENT

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REFERENCES

(1) BARRENSCHEEN, H. K., AND ALDERS, N. A. Über den Kohlenhydratestoffwechsel der ruhenden und tatigen Milchdruse. *Biochem. Z.*, **252**: 97. 1932.

(2) BORST, W. Nachweis organischer Phosphorverbindungen sowie einer Phosphatase in der Milchdruse. *Z. physiol. Chem.*, **212**: 126. 1932.

(3) CRAINE, E. M., AND HANSEN, R. G. Phosphokinase Activity of the Mammary Gland. *J. Dairy Sci.*, **37**:..... 1954.

(4) ENGELHARDT, W. A., AND LIUBIMOVA, M. N. Myosine and Adenosinetriphosphatase. *Nature*, **144**: 668. 1939.

(5) FISKE, C. H., AND SUBBAROW, Y. The Colorimetric Determination of Phosphorus. *J. Biol. Chem.*, **66**: 375. 1925.

(6) FOLLEY, S. J., AND KAY, H. D. The Alkaline Phosphomonoesterase of the Mammary Gland. *Biochem. J.*, **29**: 1837. 1935.

(7) KALCKAR, H. M. Adenylpyrophosphatase and Myokinase. *J. Biol. Chem.*, **153**: 355. 1944.

(8) LOHMANN, K. Über die Aufspaltung der Andenylpyrophosphorsaure und Argininphosphorsaure in Krebsmuskulatur. *Biochem. Z.*, **282**: 109. 1935.

(9) POEL, W. E. Preparation of Acellular Homogenates from Muscle Samples. *Science*, **108**: 390. 1948.

PHOSPHOKINASE ACTIVITY OF THE MAMMARY GLAND [1, 2, 3]

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To understand the formation of milk constituents, a more extensive knowledge of the carbohydrate metabolism of the mammary gland is essential. By use of a variety of techniques the utilization of glucose by the mammary gland has been established. Grant *(11)* and Malpress and Morrison *(15)* found that glucose was used by suitable slices of guinea pig tissue to form lactose. The latter workers obtained conversion of glucose to a nonfermentable sugar with slices of cow glands. Knodt and Petersen *(14)* noted that the perfusion of glucose through cow mammary glands increased the glycogen content of the tissue. Although there is a species variation in the gland metabolism, Folley and French *(9)* have shown that glucose is oxidized by mammary glands, since added glucose increased the oxygen consumption of mammary slices. This stimulation of the oxygen uptake by glucose was much greater with tissue from the glands of nonruminant animals in comparison to tissue from ruminant animals.

Phosphorylated intermediates would be expected to participate in lactose formation as well as in other metabolic pathways in the mammary gland. Barrenscheen and Alders *(2)* observed that added glucose depressed the breakdown of organic phosphate; this was attributed to phosphorylation of the sugar. To extend the knowledge of this subject, mammary tissue has been examined for the presence of enzymes capable of phosphorylating glucose and other sugars.

EXPERIMENTAL

Homogenates were prepared from a frozen powder of mammary tissue of lactating Holtzman rats as described previously *(7)*. The homogenizing medium was either water or 0.015 *M* potassium phosphate buffer (pH 7.4). Extracts were prepared by centrifuging the homogenates at 10,000 × G for 10 minutes.

Hexokinase was measured by disappearance of added glucose essentially as described by Vestling *et al. (21)*. For a complete system the following constituents (final molar concentration) were added in order: 0.008 *M* magnesium sulfate, 0.027 *M* potassium iodoacetate, 0.0015 *M* potassium phosphate, 0.005 *M* adenosinetriphosphate [4] (ATP), 0.05 *M* potassium fluoride, 11 micromoles of

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[2] A portion of these data was taken from a thesis presented by E. M. Craine to the Graduate Faculty of the University of Illinois to fulfill partial requirements for the degree of Doctor of Philosophy.

[3] Supported in part by a grant from the United States Atomic Energy Commission [Contract No. AT(11-1)-67, Project No. 10].

[4] The disodium salt obtained from Pabst Laboratories was neutralized with potassium hydroxide.

glucose, water to make a total volume of 2.0 ml., and 0.4 ml. of 20% homogenate. In some cases α-ketoglutarate was added. The ATP was added to initiate the action after equilibration of the incubation mixture. Incubations were performed for 40 or 60 minutes at 38° C. in Warburg flasks or small beakers. Protein and phosphate esters were removed by placing 1 ml. of incubation fluid in 2 ml. of 5% zinc sulfate; 2 ml. of 0.3 *N* barium hydroxide were added, whereon the mixture was centrifuged and filtered. After the aliquot from the incubation mixture was added to the zinc sulfate, an equivalent amount of glucose was added to the controls. The value obtained with these controls was referred to as "added glucose." Glucose was measured on the clear filtrates by the modified reduction procedure of Somogyi *(19)* using the Nelson *(17)* reagent to estimate the extent of reduction. Differences between incubated substrate values and "added glucose" are reported as micromoles of glucose esterified.

Phosphokinase activity also was measured manometrically, as described by Colowick and Kalckar *(5)*. The assay was based on the release of carbon dioxide by the acid group formed in the transfer of phosphate from adenosinetriphosphate to an acceptor. The constituents of the incubations were the same as the previous experiments except that 0.027 *M* potassium bicarbonate also was present. The Warburg flasks were equilibrated by gassing for 10 minutes with a mixture of 95% nitrogen–5% carbon dioxide; the ATP was added from a side arm of the flasks to initiate the reaction. Control flasks contained all constituents except glucose or other sugar substrate and, as previously explained *(7)*, gave high values because of apyrase activity. Incubations were performed at 38° C., usually for 60 minutes. Values given for phosphokinase are carbon dioxide released in excess of control values due to addition of substrate. All values obtained were corrected for the carbon dioxide retention of the incubation mixtures.

The sugars used were commercial analytical materials of Pfanstiehl. Each was assayed for glucose impurity by a specific glucose oxidase[5] *(13)*. There was not sufficient glucose in the fructose and in the mannose to influence the results obtained, whereas the galactose contained at a maximum 2.5% glucose. This made necessary special experiments to determine the existence of galactokinase activity. It was not possible to assay the maltose, since a maltase was present in the glucose oxidase preparation. However, the maltose was relatively free of glucose, as determined by means of paper chromatograms.

RESULTS

Glucokinase. The 4% homogenate prepared from frozen mammary tissue phosphorylates glucose so that there was a disappearance of free hexose (Table 1) in the incubation mixture. Since the reaction did not occur unless ATP was added, a transfer of phosphate from ATP was evident. The presence of fluoride favored the esterification; this was due to inhibition of apyrase which hydrolyzed ATP. Variation of the atmosphere of the flasks or the addition of α-ketoglutarate did not affect activity perceptibly.

[5] Furnished by E. M. Weber, Pfizer and Co., Inc.

TABLE 1

Glucokinase activity as measured by glucose disappearance [a]

System	Time	No. of values	Glucose esterified (mean)
	(min.)		*(micromoles)*
Complete	40	20	2.2
No ATP	40	10	0.3
No α-ketoglutarate	40	2	2.2
No α-ketoglutarate [b]	60	18	2.6
No α-ketoglutarate or fluoride	40	4	0.8

[a] Atmosphere air or oxygen with exception noted.
[b] Nitrogen atmosphere.

The esterification of glucose also was evident from the manometric assay. The results of a typical experiment using several sugars at a concentration of 11 micromoles per flask are presented in Figure 1. Glucose and mannose were phosphorylated rapidly in comparison to the other sugars. Some of the factors affecting glucokinase are summarized in Table 1. In comparing the two methods of assay with respect to phosphate buffer homogenates, it was found that about two moles of carbon dioxide were formed for each mole of glucose esterified. With low concentrations of glucose it was found that the manometric assay measured the phosphorylation of products arising from the hexokinase action; thus the glucose esterification was a more exact measure of hexokinase. Part A of Table 2 shows that homogenates prepared with phosphate buffer had more glucokinase activity than those with water. Less glucokinase activity also was found in homogenates prepared from glands containing large amounts of milk.

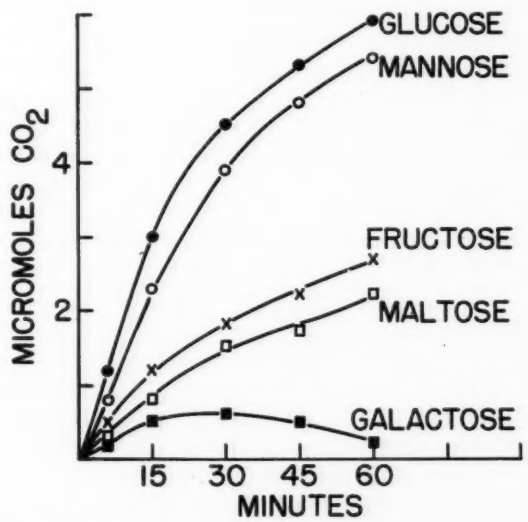

FIG. 1. Phosphorylation of sugars by mammary gland homogenate. Eleven micromoles of substrate added per Warburg cup. Final volume was 2.0 ml.

TABLE 2

Factors affecting glucokinase activity as measured manometrically

Substrate	Homogenizing medium	No. of exps.	Carbon dioxide released	
			Mean	Range
			(micromoles)	
A. Glucose	Phosphate	19	4.3	1.7 to 5.9
Glucose	Water	7	3.3	2.7 to 4.6
Glucose [a]	Phosphate	3	2.6	2.3 to 2.9
B. Glucose [b]	Phosphate	4	3.3	1.9 to 4.8
Glucose [b]	Phosphate (extract)	4	3.2	2.2 to 4.7
C. Glucose [c]	Phosphate	5	0.8	0.0 to 1.7
Glucose	Phosphate	5	3.1	2.1 to 4.0

[a] Large amounts of milk present in the tissue.
[b] Extracts and homogenates have the same source.
[c] No magnesium added.

This may have been the result of dilution of the tissue although it is recognized that other factors may be important. The extraction procedure did not influence glucokinase activity.

The addition of 0.008 *M* magnesium ions appeared to stimulate glucokinase activity (part C, Table 2) about 270% over a 60-minute period. As was previously reported *(7)*, added magnesium depressed the control values about 40%. The total carbon dioxide released was less than when magnesium was not added, but the amount in excess of the controls or that due to transphosphorylation was greater.

Other phosphokinases. As can be seen in Figure 1, several other sugars were phosphorylated by the mammary gland preparations. Of those tested, only mannose was esterified at a rate comparable to that of glucose at the 11 micromole concentration. Fructose and mannose are sufficiently free of glucose to indicate that these hexoses definitely were esterified. The galactose preparations which contained a maximum of 2.5% glucose induced kinase action of a low order.

At a concentration of 11 micromoles, glucose and mannose were phosphorylated at a maximum rate (Table 3). Concentration increases above 11 micromoles increased the rate of the reaction for maltose, fructose, and galactose. Variation in the rate was observed at low concentrations of glucose (1 to 2 micromoles). Glucose was removed from the galactose by conversion to gluconic acid by means

TABLE 3

Effect of substrate concentration on phosphokinase activity

Substrate	No. of experiments	Substrate concentration	
		11	44
		(micromoles)	
Glucose	4	3.9	3.9
Mannose	2	3.9	4.6
Fructose	4	1.7	3.6
Maltose	2	2.0	4.2
Lactose	5	0.1	0.4
Galactose	7	0.5	2.2

of glucose oxidase. When the galactose still showed activity, further experiments indicated that gluconate also was phosphorylated by mammary enzymes.

A phosphorylation of lactose (Table 3) was not observed with 11 or 44 micromoles; activity could not be induced in spite of numerous variations of the incubation conditions. It is impossible at present to say whether a series of reactions was masking a kinase action. Under the conditions for measuring the other kinases no hydrolysis of lactose occurred.

Products from a phosphorylytic or hydrolytic cleavage of maltose would produce a simulated kinase. Maltose was incubated in the absence of ATP with preparations which had strong kinase activity (Table 3). Maltose was not hydrolyzed in 30 minutes, as shown by the lack of increase of reducing power (Table 4). During 60 minutes of incubation there was no evidence of phosphorolysis since the inorganic phosphate content did not increase.

Removal of cellular debris in the extraction process increased the apparent galactokinase activity at the 11 micromole concentration (Table 5). As with glucose, water preparations were lower in activity.

TABLE 4

Incubation of maltose with mammary homogenates

After incubation	Time	Control	Maltose
		(Optical density units)	
Reducing value	15 minutes	0.22	0.22
	30 minutes	0.21	0.22
		(micromoles)	
Inorganic phosphate	60 minutes	6.9	6.8

TABLE 5

Apparent phosphorylations from the addition of a commercial preparation of galactose

Preparations	Micromoles substrate	No. of exps.	Carbon dioxide released	
			Mean	Range
			(micromoles)	
Phosphate buffer homogenates	11	7	0.5	0.2 to 1.1
Phosphate buffer homogenates	44	15	2.2	0.7 to 3.4
Water homogenates	44	11	0.9	−0.9 to 2.1
Phosphate buffer extracts	11	3	1.3	1.0 to 1.9

DISCUSSION

The data presented indicate that the mammary gland of lactating rats contains an enzyme or enzymes which are capable of transferring phosphate from ATP to a variety of sugars. Of the sugars found to be phosphorylated, glucose is probably the most important because of its predominance in the blood as the free aldose. However, large amounts of bound carbohydrate other than glucose exist in blood *(20)*; the significance of these compounds is at present unknown.

The significance of the esterification of some of the sugars is not evident. The glucose and fructose esters probably are utilized; however, the fate of the products of the mannose and maltose reactions is not certain. They may arise

only because the glucokinase lacks specificity and may not be utilized further by the tissues. Most of the studies with labeled materials *(3, 4, 10, 18)* indicate that lactose arises via glucose or a glucose derivative, although the evidence is conflicting. This would merely indicate that at some point glucose or a glucose derivative occurs in the formation of the galactose moiety of lactose. It does not eliminate the possibility that other sugars, if they are available in the blood, are converted to lactose by mammary tissue. In the guinea pig tissue slice experiments of Grant *(11)*, glucose but not mannose was converted to lactose. In contrast, Folley and French *(8)* found that mannose and glucose stimulated the oxygen uptake of rat tissue to approximately the same extent and were the only sugars tested which were capable of this action. The latter results may be due to the hexokinase activities established in this report.

The results obtained with galactose must be interpreted cautiously. The commercial galactose preparations used contain approximately 2.5% glucose. This value is based on extrapolation of oxygen consumption curves by glucose oxidase, as suggested by Keilen and Hartree *(13)*. Thus, when 11 and 44 micromoles of galactose are added, 0.2 and 1.2 micromoles of glucose are present. At these low concentrations approximately 2 micromoles of carbon dioxide arise per mole of glucose. Thus, the glucose impurity would account for the average kinase values but not for some of the individual values obtained for galactose with homogenates. In addition, it does not account for values obtained with extracts. These variations are extremely small and, if a galactose esterification occurs, it is of a small order. The attempt to purify galactose by means of glucose oxidase did not settle the point because of the ability of the mammary tissue to phosphorylate gluconate. Barkhash *(1)* has observed a hexokinase in mammary glands which would not phosphorylate galactose.

The data give no indications as to how many enzymes are involved or how specific they are. The factors affecting the phosphokinase in most cases were studied only with glucose. The differences obtained in the case of substrate concentration and extraction do not necessarily indicate existence of separate enzymes. The variation in rate as affected by concentration probably occurs because the sugars such as glucose and mannose saturate the enzyme or enzymes responsible for their phosphorylation at a relatively low substrate concentration *(16)*. The activity increases resulting from extraction probably are due to a conservation of ATP, since the removal of the cellular debris removes a large portion of the mammary apyrase activity *(7)*.

Since other animal tissue hexokinases are stimulated by magnesium *(6, 12)*, the apparent stimulation reported here may be a direct effect upon the glucokinase enzyme. However, it also may be the result of inhibition of the apyrase which thus conserves ATP and makes it more available for transphosphorylation.

SUMMARY

Mammary gland tissue homogenates phosphorylated glucose, fructose, mannose, and maltose. Glucokinase activity was stimulated by the presence of added magnesium ion. Glucose and mannose were phosphorylated at a maximum rate

at lower substrate concentrations than fructose and maltose. Phosphorylation of lactose and galactose did not occur at a significant rate. The evidence indicated that a direct phosphorylation of maltose occurred.

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REFERENCES

(1) Barkhash, A. P. Carbohydrate-Phosphorus Compounds and Enzymes of Carbohydrate Metabolism in Tissues of the Lactating Mammary Gland. *Biokhimiya*, **17**: 167. 1952.

(2) Barrenscheen, H. K., and Alders, N. A. Über den Kohlenhydratestoffwechsel der ruhenden und tatigen Milchdruse. *Biochem. Z.*, **252**: 97. 1932.

(3) Barry, J. M. Conversion of Glucose to Galactose in the Mammary Gland. *Nature*, **169**: 878. 1952.

(4) Caputto, R., and Trucco, R. A New Galactose Containing Compound from Mammary Glands. *Nature*, **169**: 1061. 1952.

(5) Colowick, S. P., and Kalckar, H. M. The Role of Myokinase in Transphosphorylations. I. The Enzymatic Phosphorylation of Hexoses by Adenyl Pyrophosphate. *J. Biol. Chem.*, **148**: 117. 1943.

(6) Colowick, S. P., Welch, M. S., and Cori, C. F. Phosphorylation of Glucose in Kidney Extract. *J. Biol. Chem.*, **133**: 359. 1940.

(7) Craine, E. M., and Hansen, R. G. Apyrase Activity of the Mammary Gland. *J. Dairy Sci.*, **37**: . 1954.

(8) Folley, S. J., and French, T. H. The Intermediary Metabolism of the Mammary Gland. 1. Respiration of Lactating Mammary Gland Slices in Presence of Carbohydrates. *Biochem. J.*, **45**: 117. 1949.

(9) Folley, S. J., and French, T. H. The Intermediary Metabolism of the Mammary Gland. 3. Acetate Metabolism of Lactating Mammary Gland Slices with Special Reference to Milk Fat Synthesis. *Biochem. J.*, **46**: 465. 1950.

(10) French, T. H., Popják, G., and Malpress, F. H. Biological Synthesis of Lactose from Carbon-14 Glucose. *Nature*, **169**: 71. 1952.

(11) Grant, G. A. The Metabolism of Galactose. II. The Synthesis of Lactose by Slices of Active Mammary Gland *in vitro*. *Biochem. J.*, **29**: 1905. 1935.

(12) Hers, H. G. Role du magnesium et du potassium dans la reaction fructokinaseque. *Biochem. et Biophys. Acta*, **8**: 424. 1952.

(13) Keilen, D., and Hartree, E. F. Properties of Glucose Oxidase (Notatin). *Biochem. J.*, **42**: 221. 1948.

(14) Knodt, C. B., and Petersen, W. E. Studies of the Carbohydrate Metabolism of Mammary Gland Tissue. I. Production and Utilization of Various Carbohydrates. *J. Dairy Sci.*, **28**: 415. 1945.

(15) Malpress, F. H., and Morrison, A. B. Studies on the Synthesis of Lactose by the Mammary Gland. I. Precursors. *Biochem. J.*, **46**: 307. 1950.

(16) Meyerhof, O., and Wilson, J. R. Studies on Glycolysis of Brain Preparations. V. Affinity of Hexokinase for Glucose and Fructose. *Arch. Biochem.*, **19**: 502. 1948.

(17) Nelson, N. A Photometric Adaption of the Somogyi Method for the Determination of Glucose. *J. Biol. Chem.*, **153**: 375. 1944.

(18) Schambye, P., Wood, H. G., and Popják, G. Synthesis of Lactose in Rabbits Studied with C^{14}-labeled Compounds. *Fed. Proc.*, **12**: 264. 1953.

(19) Somogyi, M. A New Reagent for the Determination of Sugars. *J. Biol. Chem.*, **160**: 61. 1945.

(20) Stary, Z., Bursa, F., Kaleoglu, O., and Bilen, M. Protein Bound Carbohydrate in Blood. I. Polysaccharides. *Bull. fac. med. Istanbul*, **13**: 233. 1950.

(21) Vestling, C. S., Mylroie, A. K., Irish, U., and Grant, N. H. Rat Liver Fructokinase. *J. Biol. Chem.*, **185**: 789. 1950.

A HIGH ROUGHAGE SYSTEM FOR RAISING CALVES BASED ON EARLY RUMEN DEVELOPMENT. IV. SYNTHESIS OF THIAMINE AND RIBOFLAVIN IN THE RUMEN AS INFLUENCED BY THE RATIO OF HAY TO GRAIN FED AND INITIATION OF DRY FEED CONSUMPTION

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Most of the known B-complex vitamins have been found to be synthesized by rumen microorganisms of cattle during the process of fermentation of feedstuffs *(9)*. In consideration of the natural occurrence of this symbiotic relationship in older cattle, it was reasoned that calves raised on the high roughage system, in which early rumen function is encouraged *(4, 5)*, would be able to meet their B-complex vitamin requirements at a relatively early age. That this was accomplished was indicated by the healthy appearance of the calves, their normal rate of growth, and the observation that no break occurred in the growth curve when milk was removed from the ration at 7 weeks of age *(4, 5)*.

Rumen synthesis of B-complex vitamins has been shown to be affected by the level of readily fermentable carbohydrates *(7)*, the relative amount of nitrogen in the ration *(10)*, the quality of roughage *(6, 14)*, the addition of thiamine to a ration low in B-complex vitamins, and sulfathaladine administered in therapeutic doses *(15)*. Using slaughter material, Kesler and Knodt *(8)* found that the level of B-complex vitamin synthesis did not change materially in the rumen of calves from 1 to 4 weeks of age.

In the following experiments an attempt was made to determine the extent to which riboflavin and thiamine synthesis occurred in the rumen of young calves fed on various high roughage rations and also how the synthesis of these vitamins was affected by the age at which calves began to eat dry feed.

EXPERIMENTAL PROCEDURE

Two approaches have been used by various investigators as an indication of rumen synthesis of B-complex vitamins: *(a)* measuring the level in rumen contents *(7, 10, 16)*, and *(b)* measuring the quantity excreted in urine and feces *(15)*. Measurement of the B-vitamins in the rumen contents would seem to be the most reliable method; however, this does not take into account the continuous passage of B-vitamins from the rumen. On the other hand, results obtained by using only urinary and fecal excretion may be complicated by

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variations in metabolic utilization *(2, 11)* and synthesis in the lower intestinal tract. It was therefore decided that a combination of these two methods would yield results more indicative of the actual changes occurring in thiamine and riboflavin synthesis. Accordingly, whenever possible in these experiments, thiamine and riboflavin were measured in rumen samples and also in 24-hour urine collections.

Rumen samples were obtained by stomach tube according to the method described by Pounden *(12)*. A 200- to 400-ml. sample of rumen liquor was usually obtainable by this method. In order to reduce the effect of diurnal variations, 75-ml. fractions of rumen contents were taken at three different times during the day. These samples were acidified with HCl to pH 4.0-4.5, composited, strained through two thicknesses of cheesecloth, and held under refrigeration until assayed.

Urine samples were collected during 24-hour periods by placing the calves in the metabolism stalls which have been described previously *(3)*. The urine was filtered through two thicknesses of cheesecloth and acidified with HCl in preparation for analysis.

Riboflavin was assayed microbiologically, and thiamine was determined by the thiochrome method, as described by the Association of Vitamin Chemists *(1)*.

Experiment 1. A mixture of good quality, coarsely ground, third cutting alfalfa hay (13.3% crude protein) and a simple grain concentrate mixture was fed at a 4:1, 3:2, and 2:3 ratio of hay to grain, respectively, to groups of three Jersey bull calves. The grain mixture (18.1% crude protein) contained 73% ground shelled corn and 27% soybean oil meal. Milk feeding was terminated at 7 weeks of age. Cud inoculations were given weekly for the first 6 weeks. Rumen samples were taken at 12 weeks of age along with 24-hour urine samples for thiamine and riboflavin assay. Microscopic examinations were made of the rumen samples by the indicator method described by Pounden and Hibbs *(13)*.

Two of these calves which were changed later from a 4:1 to a 2:1 ratio of hay to grain at 3 months of age were slaughtered at 6 months, and the amount of riboflavin and thiamine in the intestinal contents was measured at different levels of the alimentary tract.

Experiment 2. In this experiment the feeding schedule employed in Experiment 1 was duplicated except that good quality, chopped, second cutting mixed clover-timothy hay was used to replace the alfalfa hay. Three Jersey bull calves were used in each group for urine collections at 12 weeks of age as in Experiment 1. Rumen samples taken from seven similarly fed calves were included in each group, making a total of 10 calves in each group used for the rumen studies.

After completion of the 12-week feeding period, two of the Jersey bull calves which had been fed the 2:3 ratio of hay to grain were changed abruptly to a 4:1 ratio of hay to grain. The daily thiamine and riboflavin excretion in the urine, along with the rumen level of these vitamins, was measured and compared with results obtained prior to the change in the hay to grain ratio.

In addition, the thiamine and riboflavin content of rumen liquor was measured in single samples from 15 calves ranging in age from 5 to 43 days during the first 6 weeks of this experiment.

Experiment 3. Five Jersey bull calves which had been raised on the high roughage system were placed on a ration of mature timothy hay (6.5% crude protein) and an all-grain concentrate mixture (20% crude protein) at 3 months of age. In the first phase of this experiment, three calves were fed a 4:1 ratio of hay to grain, and two calves were placed on a 2:1 ratio. In the second phase, four of the calves were placed on different ratios of hay to grain, including all hay and all grain. Rumen samples were taken twice during the 3rd week after changing from the previous ratio. Finally, two calves were started on rations of hay alone and were given increased allowances of grain at 2-week intervals until they refused to take additional quantities of grain. Rumen samples were taken during the last week of each 2-week period. These procedures resulted in the following number of analyses for riboflavin and thiamine in the rumen juice at the various hay to grain ratios indicated: hay only, 4; 12 hay to 1 grain, 7; 6 hay to 1 grain, 5; 4 hay to 1 grain, 10; 2 hay to 1 grain, 10; 1 hay to 1 grain, 4; 0.6 hay to 1 grain, 3; and all grain, 1.

RESULTS

The results of Experiments 1 and 2, presented in Table 1, show that feeding different ratios of hay to grain (4:1, 3:2, and 2:3) did not materially affect the synthesis of thiamine. Riboflavin was increased only slightly when the narrower ratios were fed with either alfalfa or clover-timothy. The data in Table 1 also show that the calves fed alfalfa hay excreted approximately 50% less thiamine than the calves fed clover-timothy hay. The differences found in the rumen level of thiamine between types of hay were less marked and could be attributed to sample variation; therefore, it is probable that the smaller quantity of thiamine excreted in the urine of the alfalfa-fed calves was caused by factors other than decreased rumen function.

Finally, the data in Table 1 show that the dry matter of strained rumen liquor from the calves in both Experiments I and II contained four to eight times as much thiamine and riboflavin as an equal weight of the ration fed. This finding is regarded as evidence of microbial synthesis, inasmuch as the B-complex vitamins contained in rumen liquor have been found to be largely bound to the cellular material of rumen bacteria and protozoa *(2)*. Additional evidence of rumen synthesis was obtained from two calves fed a 2:1 ratio of hay to grain and slaughtered at 6 months of age (Table 2).

It was noted that when several calves were changed from a high grain ration (2:3) to a high roughage ration (4:1), they either failed to gain or declined in body weight for a period of 2 to 4 weeks. Some of these calves had depressed appetites. Therefore, the effect of an abrupt change from a 2:3 to a 4:1 ratio of hay to grain on the synthesis of thiamine and riboflavin in the rumen was studied in an effort to determine if the above symptoms might be associated with a change in rumen synthesis of these vitamins. The results of this study

TABLE 1

The effect of the ratio of hay to grain eaten on the thiamine and riboflavin content of rumen liquor and on the daily urinary excretion of these vitamins by dairy calves when either alfalfa or clover-timothy hay was used for roughage[a]

Ratio of hay to grain	Type of hay	Rumen liquor[b]		Urinary excretion[c]		Feed	
		Thiamine	Riboflavin	Thiamine[d]	Riboflavin	Thiamine	Riboflavin
		(γ/g of d.m.)	*(γ/g of d.m.)*	*(mg/d/100 lb.)*	*(mg/d/100 lb.)*	*(γ/g)*	*(γ/g)*
				Experiment 1 (3rd cutting alfalfa hay)			
4:1	alfalfa	7.6 ± 0.4	42.2 ± 10.6	0.75 ± 0.47	1.72 ± 0.14	2.00	10.71
3:2	alfalfa	9.2 ± 1.2	44.8 ± 24.1	0.90 ± 0.27	2.00 ± 0.42	2.57	8.85
2:3	alfalfa	———	———	0.87 ± 0.45	1.57 ± 0.78	2.91	6.78
				Experiment 2 (2nd cutting clover-timothy hay)			
4:1	cl.-tim.	8.5 ± 2.8	31.7 ± 8.3	2.34 ± 0.84	1.56 ± 0.09	2.14	9.80
3:2	cl.-tim.	11.2 ± 3.7	38.0 ± 11.1	1.27 ± 0.76	1.45 ± 0.84	2.54	7.80
2:3	cl.-tim.	8.9 ± 2.5	44.2 ± 24.7	1.68 ± 0.74	1.51 ± 0.55	2.88	5.16

[a] Calves were 12 to 13 weeks of age.
[b] Each value is the average and standard deviation for two calves in Experiment 1 and 10 calves in Experiment 2.
[c] Each value is the average and standard deviation for three calves in each experiment.
[d] Differences between experiments were highly significant ($P < 0.01$).

TABLE 2

The amount of thiamine and riboflavin at various levels of the alimentary tract taken from calves fed a 2:1 ratio of alfalfa hay to grain concentrate mixture[a]

Alimentary organ	Thiamine[b]	Riboflavin[b]
	(γ/g)	*(γ/g)*
Rumen	4.1	20.7
Omasum	3.6	23.2
Abomasum	2.5	26.4
Cecum	1.3	7.1
In ration fed	2.6	8.3

[a] Each value represents the average results from two calves slaughtered 6 hours after feeding.
[b] γ/g of dry matter in the contents of the alimentary tract at the various levels.

are recorded in Table 3. The average amount of thiamine per 100 lb. of body weight excreted daily in the urine dropped from 1.26 to 0.53 mg. for the first 5-day trial and to 0.31 mg. for the second 5-day trial, when the 2:3 ratio of hay to grain was abruptly changed to a 4:1 ratio. Likewise, the average rumen level of thiamine decreased from 21.5γ per 100 ml. to 8.0γ (Table 3). On the other hand, it was shown that two calves which had been fed the 4:1 ratio of hay to grain free choice from birth excreted 1.75 mg. of thiamine per day per 100 lb. of body weight and had an average of 37.0γ of thiamine per 100 ml. of rumen liquor, showing that the 4:1 ratio was capable of supporting optimum thiamine synthesis in the rumen when fed from birth with no abrupt change in feeding schedule.

Riboflavin synthesis was not observed to be affected by the abrupt change from a 2:3 to a 4:1 hay to grain ratio.

The riboflavin and thiamine content of rumen liquor in calves which were eating no dry feed at an average age of 13 days was found to have increased three- and fourfold, respectively, by the time these calves were an average of 36 days of age and were eating 0.7 lb. of dry feed per day (Figure 1). These results, based on the analysis of rumen samples from 15 calves, suggest that the ingestion of dry feed (40-80% clover-timothy hay) was the major stimulus for vitamin synthesis in the rumen of these calves. This conclusion was based on the finding that a marked increase of thiamine and riboflavin in the rumen liquor occurred simultaneously with the time that the calves began eating dry feed (Figure 1).

Microscopic examination of rumen samples from calves in Experiments 1 and 2 made by the indicator method *(13)* revealed that the indicator rumen microorganisms were rated highest in calves fed the 4:1 ratio of hay to grain and somewhat lower when the 3:2 ratio was fed. Several kinds of bacteria typically found in the rumens of mature cattle were often absent in the rumens of calves fed the 2:3 ratio.

In Experiment 3, calves were fed mature timothy hay and a simple grain concentrate mixture in ratios ranging from all hay to as much grain as the calves would consume before reaching inappetence. The data showing the levels of thiamine and riboflavin found in the rumen at the various ratios of hay to grain fed are summarized in Table 4. Thiamine was synthesized at the highest rate

TABLE 3

The influence on the urinary excretion of thiamine and riboflavin and the rumen level of these vitamins of abruptly changing the ration from a high grain to a high roughage ration

Calf No.	Trial	Ratio of hay to grain	Thiamine in rumen juice	Thiamine, daily urinary excretion	Riboflavin in rumen juice	Riboflavin, daily urinary excretion	Remarks
			(γ/100 ml.)	*(mg/100 lb.)*	*(γ/100 ml.)*	*(mg/100 lb.)*	
3	1	4:1	38.1	1.91	109.4	2.46	Calves fed the 4:1 ratio of hay to grain continuously
7	1	4:1	35.9	1.59	140.8	1.65	
Av.			37.0	1.75	125.1	2.06	
4	1	2:3	29.8	1.27	132.8	2.14	Calves fed the 2:3 ratio of hay to grain continuously through the 13th week of age
6	1	2:3	13.2	1.24	140.6	1.37	
Av.			21.5	1.26	136.7	1.76	
4	2	4:1	—	0.25	—	2.06	Urine collection made 2 to 8 days after abruptly changing from the 2:3 to 4:1 ratio of hay to grain
6	2	4:1	—	0.81	—	1.15	
Av.			—	0.53	—	1.61	
4	3	4:1	5.52	0.35	156.3	2.03	Urine collection made 9 to 14 days after abruptly changing from the 2:3 to 4:1 ratio of hay to grain
6	3	4:1	10.49	0.27	122.5	2.66	
Av.			8.01	0.31	139.4	2.35	

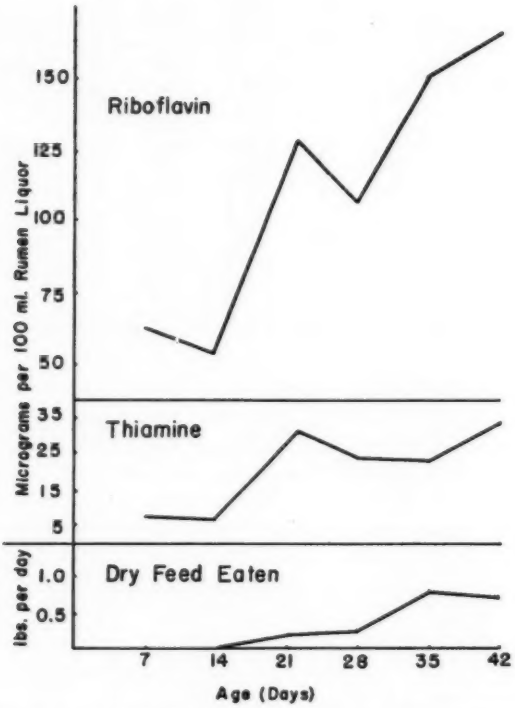

FIG. 1. The thiamine and riboflavin content in the rumen liquor of calves 6 to 42 days of age and its relation to the initiation of dry feed consumption (average daily hay and grain consumption).

when a 4:1 ratio of hay to grain was fed. The thiamine content of rumen liquor was decreased significantly ($P < 0.01$) when both wider and narrower ratios were fed.

TABLE 4

The influence of the ratio of hay to grain fed on the synthesis of thiamine and riboflavin in the rumen of calves fed mature timothy hay as the only roughage

Ratio of hay to grain	No of calves	No. of samples	Thiamine[a]		Riboflavin[a]	
			Rumen liquor	Ration fed	Rumen liquor	Ration fed
			(γ/g)	*(γ/g)*	*(γ/g)*	*(γ/g)*
All hay	2	4	2.90	1.63	15.8	4.42
12:1	3	7	2.18	1.76	17.1	4.36
6:1	3	5	4.73	1.95	21.0	4.29
4:1	5	10	10.11	2.19	30.6	4.22
2:1	5	10	6.64	2.54	49.2	4.08
1:1	3	4	5.20	2.93	46.3	4.00
0.6:1	2	3	2.60	3.07	45.4	3.90
All grain	1	1	4.06	3.94	47.4	3.58

[a] γ/g of dry matter.

Riboflavin synthesis increased as the grain concentrate increased until a ratio of 2:1 hay to grain was reached, after which further increases were not observed from feeding a higher proportion of grain (Table 4). Statistical examination of the riboflavin data disclosed significant differences between the rumen riboflavin levels in calves fed the 2:1 and the 4:1 ratios of hay to grain. Also, significant differences were found between the 4:1 ratio when compared to either the 6:1 or 12:1 ratios or the all-hay ration ($P < 0.01$).

DISCUSSION

The results reported in these experiments show that thiamine and riboflavin are synthesized in liberal quantities in the rumens of calves fed on the high roughage system (Tables 1 and 2). This confirms the postulation, so far as thiamine and riboflavin are concerned, that calves raised on the high roughage system *(4, 5)* are able to meet their B-complex vitamin needs through their symbiotic relationship with rumen microorganisms. The synthesis of riboflavin seemed to be associated with factors which would tend to promote multiplication and growth of rumen microorganisms, that is, the feeding of good quality hay, and increasing the level of readily available carbohydrates in the ration. This observation is consistent with published results which show the stimulatory effect of feeding good quality hay *(6, 14)* and of adding readily fermentable carbohydrates to the ration *(7, 10)* on the rumen synthesis of certain B-complex vitamins.

On the other hand, thiamine synthesis appeared to be influenced by still other factors. As previously indicated, it has been shown that rumen-thiamine level decreased when a high proportion of grain concentrate mixture was fed with mature timothy hay. This effect was not demonstrated where good legume hay was fed. It is possible that growth of the groups of rumen microorganisms responsible for thiamine synthesis was depressed because of the absence or the limited availability of certain nutrients required by these microorganisms when mature timothy hay was the only roughage in a high grain concentrate ration.

Another factor which appeared to limit synthesis of thiamine in the rumen was changing the ration abruptly from a 2:3 ratio of hay to grain to a 4:1 ratio. On the basis of the data presented in Table 3, the calves on the 2:3 ratio of hay to grain appeared to possess rumen microorganisms capable of synthesizing thiamine equally as well as those of calves fed the 4:1 ratio of hay to grain from birth. However, when the calves fed a 2:3 ratio of hay to grain were changed abruptly to the 4:1 ratio of hay to grain, the thiamine-synthesizing microorganisms that had existed under conditions of high grain feeding did not thrive on the high roughage ration. This postulation is supported in part by microscopic examination of rumen samples made by the indicator method *(13)*. It was shown that only a few varieties of the indicator microorganisms were found in the rumens of the calves fed the 2:3 ratio of hay to grain even though they had been cud-inoculated earlier. This was in agreement with previous findings *(13)*. Furthermore, these microorganisms did not appear in the 13-day period of observation following the abrupt change to the high roughage

ration (4:1), during which the low level of rumen thiamine content (8.0γ per 100 ml.) was observed. On the other hand, the control calves which had been raised from birth on the 4:1 ratio of hay to grain had the highest rumen thiamine content (37.0γ per 100 ml.). Also, a comparatively well balanced flora, which goes with mature type rumen function *(13)*, had become established at an earlier age as a result of rumen inoculation.

The favorable effect of the high roughage rations in stimulating the synthesis of thiamine and riboflavin at an early age is shown in Figure 1. The data indicate that rumen synthesis occurred at a relatively high rate by the time the calves were eating 0.2 lb. or more of dry feed per day or by the time the calves were approximately 3 weeks of age. This is further evidence that calves fed on the high roughage system were adequately supplied with these vitamins before milk was removed from the ration at 7 weeks of age.

Young dairy calves in which special efforts were made to develop early rumen function by encouraging consumption of good quality hay and by giving rumen inoculations using cud material proved to be useful experimental animals for studying rumen synthesis. They were particularly suitable because of the ease with which rumen samples could be taken with a stomach tube and also the ease of handling in the metabolism stalls.

SUMMARY AND CONCLUSIONS

A study was made of the effect of feeding various ratios of hay to grain with different types of hay on the synthesis of thiamine and riboflavin in the rumen of young dairy calves which had been rumen inoculated.

Composited rumen samples drawn with a stomach tube at three different times during the day and strained through cheesecloth were used as the assay material for measuring the amount of thiamine and riboflavin in the rumen liquor. The rumen liquor level of these vitamins was used, together with the daily excretion of thiamine and riboflavin in 24-hour collection periods, as an indication of rumen synthesis.

Rumen thiamine and riboflavin levels and the quantity of these vitamins excreted daily in the urine remained practically constant when different ratios of hay to grain (4:1, 3:2, and 2:3) were fed using either alfalfa or mixed clover-timothy hay with a simple grain concentrate mixture. It is concluded that rumen synthesis of these vitamins was neither augmented nor depressed by feeding different ratios of hay to grain when good quality legume or mixed legume hay was fed. In contrast, when mature timothy hay was fed, highly significant differences were observed in rumen vitamin levels between groups of calves fed different ratios. Rumen thiamine levels were maximum when a 4:1 ratio of hay to grain was fed and were minimum when either all timothy hay or a high proportion of grain concentrate mixture was fed. Rumen riboflavin content increased with increased levels of grain concentrate feeding to calves fed timothy hay until a maximum riboflavin synthesis was attained at the 2:1 ratio of hay to grain. This high rate of synthesis was maintained at higher levels of grain feeding.

Thiamine synthesis was depressed in calves which had been fed rations containing a 2:3 ratio of hay to grain when the ration was abruptly changed to a 4:1 ratio.

Results have been presented which show the favorable effect of the ingestion of dry feed (40-80% good quality clover-timothy hay) on the synthesis of thiamine and riboflavin in young rumen-inoculated calves.

The possible relationship of thiamine and riboflavin synthesis to the growth and establishment of certain varieties of rumen microorganisms when different ratios of hay to grain are fed has been discussed.

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REFERENCES

(1) Association of Vitamin Chemists, Inc. *Methods of Vitamin Assay.* Interscience Publ. Co., New York. 1951.

(2) Conrad, H. R. Some Factors which Influence the Synthetic and Digestive Processes in the Rumen of Young Dairy Calves. Dissertation, The Ohio State University, Columbus. 1952.

(3) Conrad, H. R., and Hibbs, J. W. A High Roughage System for Raising Dairy Calves Based on the Early Development of Rumen Function. III. Effect of Rumen Inoculations and the Ratio of Hay to Grain on Digestion and Nitrogen Retention. *J. Dairy Sci.*, **36**: 1326. 1953.

(4) Hibbs, J. W., Conrad, H. R., and Pounden, W. D. A High Roughage System for Raising Calves Based on the Early Development of Rumen Function. II. Growth, Feed Consumption and Utilization by Calves Fed a 3:2 Ratio of Hay to Grain with or without Molasses or Penicillin Supplement. *J. Dairy Sci.*, **36**: 1319. 1953.

(5) Hibbs, J. W., Pounden, W. D., and Conrad, H. R. A High Roughage System for Raising Calves Based on Early Development of Rumen Function. I. The Effect of Variations in the Ration on Growth, Feed Consumption and Utilization. *J. Dairy Sci.*, **36**: 717. 1953.

(6) Hunt, C. H., Bentley, O. G., and Hershberger, T. Factors Affecting the Synthesis of Certain Members of the B-Complex in an Artificial Rumen. *Federation Proc.*, **11**: 233. 1952.

(7) Hunt, C. H., Burroughs, E. W., Bethke, R. M., Gerlaugh, P., and Schalk, A. F. Studies on Riboflavin and Thiamine in the Rumen Content of Cattle. *J. Nutrition*, **25**: 207. 1943.

(8) Kesler, E. M., and Knodt, C. B. Increase in B-Vitamin Concentrations in Digestive Tract Contents of Young Dairy Calves. *Proc. Soc. Exptl. Biol. Med.*, **79**: 85. 1952.

(9) Kon, S. K., and Porter, J. W. G. The Role of the Microflora of the Alimentary Tract of Herbivora with Special Reference to Ruminants. V. Synthesis of Vitamins in Relation to Requirements. *Nutrition Abstr. and Revs.*, **17**: 31. 1947.

(10) Lardinois, C. C., Mills, R. C., Elvehjem, C. A., and Hart, E. B. Rumen Synthesis of the Vitamin B-complex as Influenced by Ration Composition. *J. Dairy Sci.*, **27**: 579. 1944.

(11) Pearson, R. B., Struglia, L., and Lindahl, I. L. The Fecal and Urinary Excretion of Certain B-Vitamins by Sheep Fed Hay and Semi-Synthetic Rations. *J. Animal Sci.*, **12**: 213. 1953.

(12) Pounden, W. D. Some Factors Affecting Rumen Function in Young Dairy Calves and Their Influence as Reflected in the Health of the Animals. Dissertation, The Ohio State University, Columbus. 1949.

(13) POUNDEN, W. D., AND HIBBS, J. W. Influence of the Ration and Rumen Inoculations on the Establishment of Certain Microorganisms in the Rumens of Young Calves. *J. Dairy Sci.*, **31**: 1041. 1948.

(14) TEERI, A. E., JOSSELYN, D., COLOVOS, N. F., AND KEENER, H. A. The Influence of the Ration on the Excretion of Certain Vitamins by Ruminants. *J. Dairy Sci.*, **34**: 1070. 1951.

(15) TEERI, A. E., LEAVITT, M., JOSSELYN, D., COLOVOS, N. F., AND KEENER, H. A. The Effect of Sulfathalidine on the Excretion of Vitamin B by Ruminants. *J. Biol. Chem.*, **182** (2): 509. 1950.

(16) WEGNER, M. I., BOOTH, A. M., ELVEHJEM, C. A., AND HART, E. B. Rumen Synthesis of the Vitamin B-Complex. *Proc. Soc. Exptl. Biol. Med.*, **45**: 769. 1940.

LACTATION RESPONSE TO ARTIFICIALLY-DEHYDRATED AND PELLETED ALFALFA ADDED TO A RATION OF TIMOTHY-GRASS MIXED HAY AND GRAIN[1]

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Adequate artificial dehydration of alfalfa results in a minimum loss of nutrients and in a feed almost equivalent in value to that found at the time of cutting *(8, 11)*. The ad libitum feeding of artificially-dehydrated and pelleted alfalfa as the sole source of roughage was found to be superior to field-cured and field-baled alfalfa for the young dairy calf *(6)* but not for the lactating cow *(15)*. A reduction in fat percentage occurred when the pelleted alfalfa was fed *(15)* and, in one trial out of two, diminished feed intake was accompanied by decreases in yield of fat-corrected milk. Artificially-dehydrated and pelleted alfalfa fed as a grain replacement at the 30% level was superior to finely ground artificially-dehydrated alfalfa for milk production *(3)*. The addition of 0.5 lb. of artificially-dehydrated and pelleted alfalfa per 100 lb. of live weight to a ration of alfalfa or prairie hay fed ad libitum, sorgo silage based on body weight, and grain based on milk production resulted in significant increases in hay equivalent consumed per cow per day and in fat-corrected milk *(2)*.

The objective of the present experiment was to determine the lactation response to graded levels of artificially-dehydrated and pelleted alfalfa when added to a basal ration of timothy-grass mixed hay fed ad libitum and grain fed on the basis of milk production.

EXPERIMENTAL

Animals. From December, 1951, through July, 1952, 12 Holstein and 12 Guernsey cows were divided into six groups of four each. The cows were balanced with respect to breed, and within each breed group, in so far as possible,

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TABLE 1

Age, previous lactations, stage of lactation, and stage of gestation of experimental groups

Expt. group Art.-dehydrated and pelleted alfalfa fed	Years of age at start of experiment	Previous lactations	Days in lactation at start of experiment	Days in gestation at end of experiment
(Lb/100 lb. live weight/cow/day)				
0.0	3.4 ± 0.4	1.0 ± 0.3	60 ± 10	99 ± 14
0.5	3.8 ± 0.6	1.3 ± 0.6	45 ± 6	60 ± 25
1.0	4.1 ± 0.6	1.2 ± 0.5	56 ± 5	103 ± 14
1.5	3.5 ± 0.4	0.8 ± 0.3	51 ± 10	61 ± 22

with respect to age and previous number of lactations (Table 1). The animals within a group were assigned to one of four different treatments according to a previously randomized allotment. These consisted of the addition of one of four levels, 0.0, 0.5, 1.0, 1.5 lb. per 100 lb. live weight per day, of artificially-dehydrated and pelleted alfalfa to a basal ration for a 100-day period. Prior to the addition of the assigned level of the artificially-dehydrated and pelleted alfalfa (comparison period), each cow was fed the basal ration alone for a 30-day standardizing period. The standardizing period was used for subsequent adjustment of individual cow differences by regression methods.

Ration. The basal ration consisted of timothy-grass mixed hay fed ad libitum, and grain fed at the rate of 1 lb. per 4 lb. of fat-corrected milk during the standardizing period and 1 lb. to 5 lb. during the comparison period. The average U. S. grade of the timothy-grass mixed hay was 1.7, and the botanical composition was 7.7% foreign matter, with the remainder made up of 51.7% timothy, 14.5% clover and alfalfa, and 33.4% other grasses. The grain mixture contained per ton: 600 lb. crimped oats, 400 lb. hominy feed, 320 lb. wheat bran, 340 lb. linseed oil meal, 200 lb. molasses, 40 lb. salt, and 40 lb. steamed bone meal.

The artificially-dehydrated and pelleted alfalfa was produced and processed during October, 1951, in Dawson County, Nebraska, and purchased on the open market. The pellets were cylindrical in shape, 1.8 cm. in diameter, and averaged 1.12 ± 0.02 cm. in length. A 200-g. sample of these pellets was covered with water in an evaporating dish, dried in an oven at 100° C., allowed to come to constant weight at room temperature, and then passed through a series of U. S. standard sieves to estimate particle size. Of this sample, 7.9% was retained by Sieve No. 12, 60% by No. 40, 19.7% by No. 60, 8.4% by No. 80, and 2.9% by No. 100, with 1.1% passing through all sieves. The average chemical composition of the feeds is given in Table 2.

The timothy-grass mixed hay was fed in sufficient amounts to provide a 5 to 10% weighback per cow per day. For the purpose of calculating the grain and artificially-dehydrated and pelleted alfalfa allowances for each cow, both the standardizing and comparison periods were subdivided into 10-day periods. The daily grain allotment for each cow was recalculated at the end of each 10-day period from average milk production data for the two previous 10-day periods. Artificially-dehydrated and pelleted alfalfa allotments for each cow were recalcu-

TABLE 2
The mean chemical composition of feeds

Feed	Composition						
	Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash	Carotenoids
	(%)	*(% of the dry matter)*					*(mg/lb)*
Timothy grass mixed hay—fed	91.9 ±0.5	7.7 ±0.6	1.7 ±0.2	33.6 ±1.3	50.6 ±0.8	6.4 ±0.7	4.70 ±1.40
Timothy grass mixed hay—refused	91.5 ±0.6	8.1 ±0.3	1.7 ±0.1	35.4 ±1.3	48.8 ±0.9	6.0 ±0.4	3.37 ±0.85
Grain mixture	90.7 ±0.5	16.9 ±0.4	4.6 ±0.2	7.8 ±0.2	62.7 ±0.6	8.0 ±0.3	0.12 ±0.07
Artificially-dried and pelleted alfalfa	92.5 ±0.4	25.6 ±0.3	4.0 ±0.1	19.2 ±0.5	39.5 ±0.5	11.7 ±0.3	70.53 ±6.22

lated at the end of each 10-day period from the average of the live weights for the first and 10th days of the previous period.

Observations and analyses. All feeds fed and refused and milk yields for each cow were weighed daily to the nearest 0.1 lb. both A.M. and P.M. Individual live weights to the nearest pound were obtained on the first and 10th day of each 10-day period. Samples of feed fed and refused were obtained at successive 28-day intervals from composited daily samples for the duration of the experiment. Aliquot samples of the A.M. and P.M. milkings for each cow for each 10-day period were composited for subsequent fat determinations. Also, similar samples were collected on the 20th and 30th days of the standardizing period and on the 25th, 50th, 75th, and 100th days of the comparison period for carotenoid and vitamin A analysis, which included fat and specific gravity determinations.

The proximate composition of the feed samples was determined by the standard A.O.A.C. procedures *(1)*; the carotenoid content of the feeds by the method of Moore and Ely *(12)*, using a mixture of starch-supercel as the chromatographic absorbent; the fat content of the milk by the A.O.A.C. Babcock procedure *(1)*; the fat-corrected milk (FCM) by the formula of Gaines *(7)*; the carotenoid and vitamin A content of the milk by the method of Boyer *et al. (4)*; and the specific gravity by a standard procedure *(1)*. The total digestible nutrient (TDN) contents of the timothy-grass mixed hay were derived according to the method of Schneider *et al. (18)* from the actual proximate composition and the digestibility coefficients for timothy-grass mixed hay as listed by Schneider *(16)*. The TDN contents of the grain mixture and of the artificially-dehydrated and pelleted alfalfa were calculated by the method of Schneider *et al. (17)* from their respective proximate compositions. Net energy (NE) calculations were derived from the TDN by the formula of Moore *et al. (13)*, which utilized Forbes' data. Based on these calculations, the timothy-grass hay as fed contained 54.7 lb. of TDN per 100 lb. of dry matter; the timothy-grass hay as refused, 54.4; the grain, 65.7; and the artificially-dehydrated and pelleted

alfalfa, 68.1. Similar values for therms of NE per 100 lb. of dry matter were, respectively, 40.7, 40.3, 56.7, and 60.2.

The statistical analysis *(5)* considered the variability among groups of cows and among levels of artificially-dehydrated and pelleted alfalfa fed and the interaction of breed times level of alfalfa fed. The variability due to levels of alfalfa was further explored with respect to linear and quadratic trends and deviations from these trends. When accounting for significant ($P < 0.10$) reduction in the sums of squares, the data for the comparison period were adjusted for that of the standardizing period. The values in the tables are arithmetic means plus or minus their respective standard errors.

RESULTS AND DISCUSSION

Feed consumed. The addition of each level of artificially-dehydrated and pelleted alfalfa resulted in a decrease ($P < 0.01$) in the ad libitum daily consumption of the timothy-grass mixed hay (Table 3). In contrast, daily grain fed and dry matter, TDN, and NE consumed increased ($P < 0.001$). Investigation of the rates of these changes on levels of pelleted alfalfa added indicated that they were essentially linear ($P < 0.001$). When utilizing the adjusted values, these changes per 0.5 lb. of the pelleted alfalfa added per 100 lb. of live weight were -1.6 ± 0.1 lb. per cow per day for the timothy-grass hay, +0.9

TABLE 3

Effect of adding graded levels of artificially-dehydrated and pelleted alfalfa on feed consumption of lactating cows

Criterion	Pounds of artificially-dehydrated and pelleted alfalfa fed per 100 lb. live weight/cow/day			
	0.0	0.5	1.0	1.5
Timothy-grass mixed hay		*(lb/cow/day)*		
Standardizing period	26.6 ± 2.0	26.7 ± 3.6	28.8 ± 3.2	28.1 ± 3.7
Comparison period	22.5 ± 1.8	22.1 ± 2.9	21.7 ± 1.4	18.7 ± 2.0
Adjusted values[a]	23.2	22.8	20.8	18.3
Grain mixture				
Standardizing period	7.1 ± 0.9	7.9 ± 1.1	8.0 ± 1.1	8.8 ± 0.9
Comparison period	4.7 ± 0.6	6.1 ± 0.6	6.8 ± 0.9	7.5 ± 0.8
Art.-dehydrated and pelleted alfalfa				
Comparison period	0.0 ± 0.0	5.7 ± 0.6	10.8 ± 0.6	16.8 ± 1.4
Dry matter				
Standardizing period	31.3 ± 2.5	32.0 ± 4.2	34.2 ± 3.9	34.1 ± 4.1
Comparison period	24.7 ± 2.1	31.1 ± 3.7	36.3 ± 2.7	39.7 ± 3.8
Adjusted values[a]	26.2	32.0	35.1	38.5
Total digestible nutrients				
Standardizing period	17.8 ± 1.4	18.3 ± 2.4	19.5 ± 2.2	19.6 ± 2.3
Comparison period	14.0 ± 1.2	18.4 ± 2.2	21.9 ± 1.6	24.6 ± 2.3
Adjusted values[a]	14.9	18.8	21.2	24.0
Net energy		*(therms/cow/day)*		
Standardizing period	13.8 ± 1.1	14.2 ± 1.8	15.1 ± 1.7	15.2 ± 1.8
Comparison period	10.8 ± 0.9	14.6 ± 1.7	17.7 ± 1.3	20.3 ± 1.9
Adjusted values[a]	11.5	15.0	17.2	19.7

[a] Adjusted value represents the comparison period value adjusted for standardizing period value by regression methods *(5)* to equalize insofar as possible individual cow differences.

$\pm$ 0.1 for the grain (unadjusted), $+4.0 \pm 0.3$ lb. for the dry matter, $+3.0 \pm 0.2$ lb. for the TDN, and $+2.7 \pm 0.1$ therms for the NE. These results agree with those of Bartley *et al. (2)* but extended the limits of their study, 0.5 lb. of pelleted alfalfa, to the 1.5 lb. level.

Milk production. Both average daily milk production and average daily FCM (Table 4) increased ($P < 0.01$) with each increment of pelleted alfalfa added to the ration. The fat percentage of the milk appeared not to be affected. The adjusted FCM per 0.5 lb. of alfalfa added per 100 lb. of live weight increased $+2.7 \pm 0.4$ lb. per cow per day. The adjusted average daily yield on the basal ration alone during the 100-day comparison period was 76.6% of the average yield during the 30-day standardizing period. When 0.5 lb. of pelleted alfalfa was added per 100 lb. of live weight, the percentage was 85.2; for the 1.0 lb. level, 96.4; and for the 1.5 level, 99.4.

The carotenoid concentration of the milk increased almost directly with each added level of the pelleted alfalfa, $5 \pm 1\gamma$ % ($P < 0.001$). Although similar increases were found for the vitamin A concentration, $1.3 \pm 0.1\gamma$ % ($P < 0.05$), this was not entirely a direct relationship. At the higher levels of alfalfa the increase in vitamin A concentration was not as great as at the lower levels ($P < 0.10$ for the quadratic trend). The adjusted concentrations of carotenoids and of vitamin A in γ per gram of fat were 6.0, 3.91; 7.8, 4.52; 9.1, 4.70; and 9.9, 4.90, respectively, for the 0.0, 0.5, 1.0, and 1.5 levels of artificially-dehydrated

TABLE 4

Effect of adding graded levels of artificially-dehydrated and pelleted alfalfa on milk production and carotenoid and vitamin A content of milk

Criterion	Pounds of artificially-dehydrated and pelleted alfalfa fed per 100 lb. live weight/cow/day			
	0.0	0.5	1.0	1.5
Av. daily lb. milk/cow				
Standardizing period	31.8 $\pm$ 5.1	34.3 $\pm$ 4.5	36.8 $\pm$ 4.8	38.3 $\pm$ 4.1
Comparison period	22.2 $\pm$ 3.9	28.9 $\pm$ 3.5	34.5 $\pm$ 4.5	37.4 $\pm$ 4.5
Adjusted values[a]	25.6	29.9	33.1	34.5
Av. fat %/cow/10 d. period				
Standardizing period	3.7 $\pm$ 0.7	3.9 $\pm$ 0.1	3.7 $\pm$ 0.1	3.8 $\pm$ 0.2
Comparison period	4.0 $\pm$ 0.3	4.0 $\pm$ 0.2	3.9 $\pm$ 0.1	4.0 $\pm$ 0.2
Adjusted values[a]	4.0	3.9	3.9	4.0
Av. daily lb. FCM/cow				
Standardizing period	29.3 $\pm$ 3.6	33.3 $\pm$ 3.8	35.0 $\pm$ 4.6	37.3 $\pm$ 3.8
Comparison period	21.4 $\pm$ 3.0	28.3 $\pm$ 2.8	33.7 $\pm$ 4.0	37.1 $\pm$ 4.0
Adjusted values[a]	25.8	28.7	32.5	33.5
Carotenoid content in γ/100 ml. milk				
Standardizing period	28 $\pm$ 7	27 $\pm$ 5	30 $\pm$ 6	26 $\pm$ 5
Comparison period	27 $\pm$ 8	32 $\pm$ 6	42 $\pm$ 9	40 $\pm$ 8
Adjusted values[a]	26	32	39	42
Vitamin A content in γ/100 ml. milk				
Standardizing period	16.6 $\pm$ 1.9	19.2 $\pm$ 1.8	19.0 $\pm$ 1.6	16.6 $\pm$ 2.0
Comparison period	15.4 $\pm$ 1.3	18.6 $\pm$ 2.7	20.0 $\pm$ 1.3	19.1 $\pm$ 2.0

[a] Adjusted value represents the comparison period value adjusted for standardizing period value by regression methods *(5)* to equalize insofar as possible individual cow differences.

and pelleted alfalfa. These were found to be directly related to level of alfalfa ($P < 0.001$ for carotenoids and $P < 0.05$ for vitamin A).

The data on milk production essentially agree as to trend with those of Bartley *et al. (2)*; however, the absolute responses were different. For example, Bartley *et al.* observed only a 0.9 lb. increase in FCM in the first trial and 1.2 lb. in the second for the 0.5 lb. level pelleted alfalfa, whereas that observed here for the same comparison was 2.9 lb. In terms of carotenoid and vitamin A concentrations, Bartley *et al.* reported increases amounting to 11γ and 0.2γ, respectively, whereas those found here were 6γ and 3.2γ, respectively. In considering these responses in milk production, the differences in the two types of basal rations, alfalfa or prairie hay plus sorgo silage versus timothy-grass mixed hay, and possible differences in the pelleted alfalfa need to be kept in mind. In the experiment reported herein, a very early cut alfalfa was artificially-dehydrated and pelleted. This resulted in a product with considerable higher calculated TDN (68.1%) and NE (60.2 therms) than that reported in the literature *(14, 16)*. In the application of the results, these variable factors should be considered.

Live weight. Changes in live weight (Table 5) appeared to be related to the level of pelleted alfalfa added to the basal ration, for there was a calculated increase of 20 ± 5 lb. ($P < 0.05$) for each 0.5 lb. of pelleted alfalfa added per 100 lb. of live weight. The rate of change with each added level of pelleted alfalfa was not entirely linear, for the cows fed the 1.5 lb. level of pelleted alfalfa increased in live weight approximately the same amount as those on the 0.5 lb. level, resulting in a quadratic relationship for all levels ($P < 0.05$).

Efficiency of feed utilization. Maintenance requirements were calculated for each cow as based on the average live weight for the standardizing period and for the comparison period; these were at the rate of 7.5 lb. TDN or 6.0 therms

TABLE 5

Effect of adding graded levels of artificially-dehydrated and pelleted alfalfa on live weight changes and utilization of feed for milk production

Criterion	Pounds of artificially-dehydrated and pelleted alfalfa fed per 100 lb. live weight/cow/day			
	0.0	0.5	1.0	1.5
Live weight in lb.				
Av. for standardizing period	1012 ± 63	1117 ± 110	1054 ± 80	1141 ± 89
Change[a]	−40 ± 16	+12 ± 8	+49 ± 24	+15 ± 11
Lb. TDN/lb FCM[b]				
Standardizing period	.354 ± .013	.303 ± .031	.335 ± .020	.300 ± .034
Comparison period	.307 ± .022	.358 ± .031	.423 ± .025	.446 ± .034
Adjusted values[c]	.265	.386	.407	.477
Therms/lb FCM[b]				
Standardizing period	.266 ± .010	.229 ± .023	.252 ± .015	.228 ± .025
Comparison period	.224 ± .016	.284 ± .024	.346 ± .020	.374 ± .028
Adjusted values[c]	.191	.306	.333	.398

[a] Difference between av. for standardizing period and end of comparison period.

[b] Lb. of TDN and therms represent that available for milk production after subtraction from each cow's total intake each cow's average maintenance requirement during the indicated period.

[c] Adjusted value represents the comparison period value adjusted for standardizing period value by regression methods *(5)* to equalize insofar as possible individual cow differences.

of NE per 1,000 lb. of live weight *(14)* adjusted to 0.75 power of live weight *(10)*. Subtracting the maintenance requirement from the total daily TDN or therm intakes, the amount of these constituents available for milk production, and dividing the difference by the average daily production of FCM gave the pounds of TDN or therms of NE utilized to produce each pound of FCM. As can be seen in Table 5, for each 0.5 lb. of pelleted alfalfa added, the amount of TDN or NE used per pound of FCM was increased ($P < 0.001$). This relationship was linear and for the adjusted values amounted to $+0.066 \pm 0.015$ lb. TDN and $+0.065 \pm 0.013$ therms per pound of FCM for each 0.5 lb. of pelleted alfalfa added to the basal ration. These data agreed with the estimates of Morrison *(14)* on the TDN and NE requirements to produce a pound of FCM and with the observation of several experiment stations, as summarized by Jensen *et al. (9)*, that on relatively high levels of nutritive intake cows are generally less efficient in production of milk than at relatively low levels.

Interaction of breed times level of pelleted alfalfa. With the exception of vitamin A concentration of the milk, no significant interaction of breed times level of pelleted alfalfa was found for any of the other criteria studied. In vitamin A concentration of the milk, Holsteins did not increase at as great a rate with increasing levels of pelleted alfalfa as did Guernseys ($P < 0.05$).

SUMMARY

The lactation response to the addition of graded levels of artificially-dehydrated and pelleted alfalfa to a basal ration of timothy-grass mixed hay fed ad libitum and grain fed on the basis of 1 lb. per 5 lb. of FCM was studied in a 100-day trial. Twelve Guernsey and 12 Holstein cows whose average daily production of FCM and average live weight during a 30-day base period were 33.7 $\pm$ 1.9 lb. and 1083 $\pm$ 42 lb., respectively, were used. For each addition to the basal ration of 0.5 lb. of pelleted alfalfa from 0.0 through 1.5 lb. per 100 lb. of live weight, the following changes were found:

1. The ad libitum consumption of timothy-grass mixed hay decreased 1.6 $\pm$ 0.1 lb. per cow per day.
2. The grain fed increased 0.9 $\pm$ 0.1 lb. per cow per day.
3. The dry matter, TDN, and NE intakes increased 4.0 $\pm$ 0.3 lb., 3.0 $\pm$ 0.2 lb., and 2.7 $\pm$ 0.1 therms per cow per day, respectively.
4. The FCM increased 2.7 $\pm$ 0.4 lb. per cow per day.
5. The carotenoid and vitamin A concentration in the milk increased by 5 $\pm$ 1 and 1.3 $\pm$ 0.1γ%, respectively.
6. The live weight increased 0.20 $\pm$ 0.05 lb. per cow per day.
7. The pounds of TDN and NE required to produce a pound of FCM after accounting for maintenance requirements increased 0.066 $\pm$ 0.015 lb. and 0.065 $\pm$ 0.013 therms, respectively.
8. With the exception of vitamin A concentration of the milk, for which Guernseys increased at a greater rate than did Holsteins, no significant breed difference in response was observed.

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REFERENCES

(1) Association of Official Agricultural Chemists. *Methods of Analysis.* 7*th* ed. A.O.A.C., Washington, D. C. 1950.

(2) Bartley, E. E., Parrish, D. B., Fountaine, F. C., and Whitnah, C. H. Effects of Supplementing Dairy Cow Rations with Dehydrated Alfalfa (Abs.). *J. Dairy Sci.*, **34**: 509. 1951.

(3) Blosser, T. H., Murdock, F. R., Lintott, R. E., Erb, R. E., and Shaw, A. O. The Use of Dehydrated Forages in Dairy Cattle Rations. II. Comparative Values of Finely Ground, Chopped and Pelleted Dehydrated Alfalfa as Grain Replacements for Lactating Dairy Cows. *J. Dairy Sci.*, **35**: 515. 1952.

(4) Boyer, P. D., Spitzer, R., Jensen, C., and Phillips, P. H. Determination of Vitamin A and Carotene in Milk. A Rapid Extraction Procedure. *Ind. Eng. Chem., Anal. ed.*, **10**: 101. 1944.

(5) Cochran, W. G., and Cox, Gertrude M. *Experimental Designs.* John Wiley and Sons, Inc., New York. 1950.

(6) Eaton, H. D., Dolge, K. L., Mochrie, R. D., Avampato, J. E., and Moore, L. A. Field-cured and Field-baled Alfalfa Hay *versus* Artificially-dried and Chopped and Pelleted Alfalfa Hays as a Source of Carotene and Roughage for Guernsey and Holstein Calves. *J. Dairy Sci.*, **35**: 98. 1952.

(7) Gaines, W. L., and Davidson, F. A. Relation Between Percentage Fat Content and Yield of Milk. Ill. Agr. Expt. Sta., *Bull.* 245. 1923.

(8) Huffman, C. F. Roughage Quality and Quantity in the Dairy Ration, A Review. *J. Dairy Sci.*, **22**: 889. 1939.

(9) Jensen, E., Klein, J. W., Rauchenstein, E., Woodward, T. E., and Smith, R. H. Input-Output Relationships in Milk Production. U.S.D.A., *Tech. Bull.* 815. 1942.

(10) Kleiber, Max. Body Size and Metabolic Rate. *Physiol. Revs.*, **27**: 511. 1947.

(11) Monroe, C. F., Hilton, J. H., Hodgson, R. E., King, W. A., and Krauss, W. E. The Loss of Nutrients in Hay and Meadow Crop Silage During Storage. *J. Dairy Sci.*, **29**: 239. 1946.

(12) Moore, L. A., and Ely, Ray. Extraction of Carotene from Plant Material. A Rapid Quantitative Method. *Ind. Eng. Chem., Anal. ed.*, **13**: 600. 1941.

(13) Moore, L. A., Irvin, H. M., and Shaw, J. C. Relationship Between TDN and Energy Values of Feeds. *J. Dairy Sci.*, **36**: 93. 1953.

(14) Morrison, F. B. *Feeds and Feeding.* 21*st* ed. Morrison Pub. Co., Ithaca, N. Y. 1948.

(15) Porter, G. H., Johnson, R. E., Eaton, H. D., Elliott, F. I., and Moore, L. A. Relative Value for Milk Production of Field-cured and Field-baled, Artificially Dried-chopped, Artificially Dried-ground and Artificially Dried-pelleted Alfalfa when Fed as the Sole Source of Roughage to Dairy Cattle. *J. Dairy Sci.*, **36**: 1140. 1953.

(16) Schneider, B. H. *Feeds of the World, Their Digestibility and Composition.* Agr. Expt. Sta., W. Va. Univ., Morgantown. 1947.

(17) Schneider, B. H., Lucas, H. L., Cipolloni, Mary A., and Pavlech, Helen M. The Prediction of Digestibility for Feeds for which There Are only Proximate Composition Data. *J. Animal Sci.*, **11**: 77. 1952.

(18) Schneider, B. H., Lucas, H. L., Pavlech, Helen M., and Cipolloni, Mary A. Estimation of the Digestibility of Feeds from Their Proximate Composition. *J. Animal Sci.*, **10**: 706. 1951.

ASPARTATE DISSIMILATION REACTIONS OF RUMEN BACTERIA[1]

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Recently, El-Shazly *(5)* reported observations on the conversion of amino acids to ammonia and fatty acids in the rumen. Sirotnak *et al. (9)*, in a study of amino acid dissimilation by washed suspensions of bovine rumen bacteria, demonstrated the formation of ammonia, carbon dioxide and acetate propionate and butyrate from L-aspartate. From the data presented at that time, it was tentatively concluded that an aspartate decarboxylase was not involved in these reactions. Carbon dioxide was believed to be evolved from the decarboxylation of a C_4 intermediate produced by a prior deamination of aspartate. Among the evidence marshalled to support this view, the following points are emphasized: *(a)* Ammonia and carbon dioxide production varies directly with increase or decrease in pH from the optimum of 6.9. *(b)* The stimulation of ammonia production by a fermentable carbohydrate is invariably accompanied by an increase in carbon dioxide production. The consistent parallelism observed between ammonia and carbon dioxide production tends to rule out the possibility of a decarboxylase, in addition to a deaminase, attacking aspartate. Such parallelism is thought to result solely from the dependence of the decarboxylation reaction upon the necessarily prior deamination.

In this paper, results of experiments designed to test the hypothesis that the degradation of aspartate proceeds via a C_4 intermediate are presented. In addition, attempts to chart the pathway by which the C_4 compound is converted to fatty acids are described.

EXPERIMENTAL

Rumen samples were collected and processed according to the methods previously given by Doetsch *et al. (2)*. Manometric techniques also were essentially the same. Five micromoles of substrate and 1 ml. of mixed cell suspension or an equivalent amount of cell-free extract were incubated at 39° C. in standard Warburg flasks for a period not exceeding 2 hours. With one exception, all reactions were complete within the 2-hour period. Dissimilation tests, designed for determining the amount of fatty acids produced from 150 μM of substrate, were similar to those outlined previously by Sirotnak *et al. (9)*.

Ammonia determination was made colorimetrically by the method of Conway *(1)*. Fatty acids were determined chromatographically by the method of Elsden *(4)*. This procedure was modified by substituting the "alphamine Red R" indicator of Liddel and Rydon *(8)*.

The preparation of the cell-free extracts was carried out by hand grinding in a manner outlined by Umbreit *et al. (11)*. However, activated alumina (Alcoa) was used as the abrasive instead of powdered glass.

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RESULTS

Results obtained on the production of carbon dioxide from succinate, malate, fumarate, oxaloacetate, pyruvate, lactate, L-aspartate, α-alanine, and β-alanine, by using intact cells and cell-free extracts, are presented in Table 1. Data on ammonia production from amino acids also are given.

TABLE 1

The decomposition of selected substrates by mixed suspensions of rumen bacteria and cell-free extracts as measured manometrically

Substrate[a]	Intact cells					Cell-free extracts		
	Total gas produced	Total CO_2 produced	CO_2 mole	$Q^{N_2}_{CO_2}$	NH_3 produced	Total CO_2 produced	$Q^{N_2}_{CO_2}$ Calculated	NH_3 produced
	(μl.)	*(μl.)*	*(g.)*		*(μM)*	*(μl.)*		*(μM)*
Aspartate	112	112	1.00	7.8	4.9	74	30.8	3.0
Succinate	108	108	0.96	10.0		68	29.0	
Malate	146	146	1.30	15.6		112	46.0	
Fumarate	105	105	0.94	15.0		90	37.0	
Oxaloacetate	0	0	0.0	0.0		0	0.0	
Pyruvate	114	86	0.76	8.2		54	23.0	
Lactate	22	14	....[b]	1.6		7	3.0	
α-alanine	0	0	0.0	0.0	0.0	0	0.0	0.0
β-alanine	0	0	0.0	0.0	0.0	0	0.0	0.0

[a] 5 μM of substrate were incubated at 39° C. for 2 hours.
[b] Reaction not completed during experimental time.

Succinate, fumarate, and pyruvate were found to be similar to aspartate in respect to total gas production (105-112 μl.). Values obtained for malate were significantly higher (146 μl.). No gas was produced from oxaloacetate, α-alanine, or β-alanine and only a small amount (22 μl.) from lactate. In contrast to aspartate, succinate, malate, and fumarate, both pyruvate and lactate yielded appreciable amounts of gas other than carbon dioxide. Approximately 1 mole of carbon dioxide was produced per mole of aspartate, succinate, and fumarate. The ratio was higher for malate (1.3) and lower for pyruvate (0.76).

A similar relationship existed among the substrates tested when cell-free extracts were employed, although respective values for total carbon dioxide production were somewhat lower. Substrate dissimilation occurred at a more rapid rate. However, no activity was observed after 20-minute incubation. This might be expected, since a partial destruction of some essential constituent probably occurs during preparation of the extracts. This is especially true if the constituent is the type that is not regenerated during the reaction.

The rates of decomposition of succinate, malate, fumarate, and pyruvate were greater than the rate obtained with aspartate. Whereas aspartate was actively deaminated by both intact cells and cell-free extracts, α-alanine and β-alanine were not.

Fatty acid production from all the substrates included in this study was determined by the dissimilation method. Analysis was made after a 5-hour incubation period before any appreciable change in the proportion of fatty

acids occurred as the result of eventual interconversion. The data, expressed as molar per cent ratios, are listed in Table 2.

No detectable fatty acid production was observed from fumarate, oxaloacetate, lactate, α-alanine, or β-alanine. The production of fatty acids from succinate was similar to that obtained from aspartate. Both substrates were converted mainly to propionate (83-100%) during the 5-hour incubation period. Malate and pyruvate were converted mostly to acetate, 55 and 69% respectively.

TABLE 2

Fatty acid production from selected substrates by mixed suspensions of rumen bacteria

Substrate[a]	Molar per cent ratio of fatty acids		
	Acetate	Propionate	Butyrate
L-aspartate	0.0	10.0	0.0
Succinate	1.0	8.3	0.7
Malate	5.5	3.7	0.8
Fumarate	0.0	0.0	0.0
Oxaloacetate	0.0	0.0	0.0
Pyruvate	6.9	1.5	1.6
Lactate	0.0	0.0	0.0
α-alanine	0.0	0.0	0.0
β-alanine	0.0	0.0	0.0

[a] 150 μM of substrate were incubated at 39°C. for 5 hours.

With one exception, butyrate production was low or absent where fatty acids were produced. A more significant amount of butyrate (16%) was formed from pyruvate.

The use of 0.3 *M* malonate for the inhibition of succinate decarboxylation as proposed by Delwiche *(3)* appeared to offer a means of determining the relationship of succinate in the pathway of aspartate dissimilation. Use of this compound (0.3 *M* malonate) resulted in an 80-90% inhibition of carbon dioxide production from both succinate and aspartate. Unfortunately, however, malate dissimilation also was inhibited. In order that some differentiation between malate and succinate inhibition could be obtained, different concentrations of malonate were employed in manometric experiments. The results are shown in Figure 1. A significant inhibition of aspartate and succinate decarboxylation occurred at a concentration of 0.03 *M* malonate. Inhibition of malate decarboxylation did not become significant until the concentration of malonate reached 0.15 *M*. With aspartate the ammonia values plotted were somewhat higher than corresponding values for carbon dioxide.

It was originally observed that the kind and amount of fatty acids formed from aspartate were determined in part by the time of incubation. An analysis of the fermentation liquid at various intervals was made in order to determine the sequence in the formation of propionate, acetate, and butyrate (Figure 2). For a relatively short time after incubation (5 hours) the fatty acids consisted mainly of propionate (95-100%). The per cent propionate gradually decreased as incubation proceeded. At the same time, the per cent acetate increased. Butyrate formation was detected last, at a point when the amount of acetate

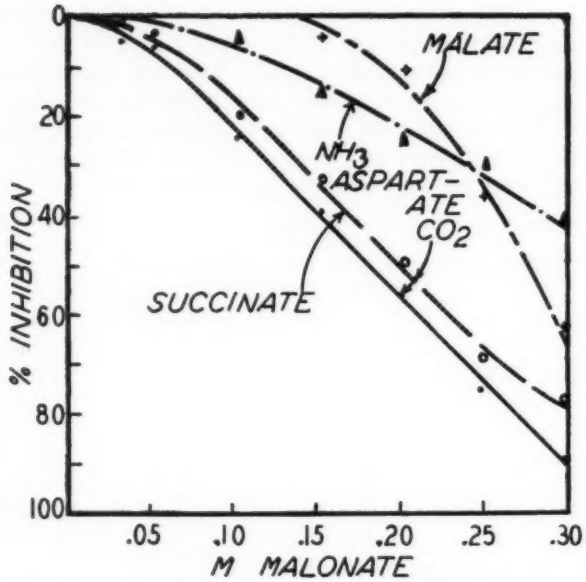

FIG. 1. Inhibition of aspartate, succinate, and malate decarboxylation by various concentrations of malonate.

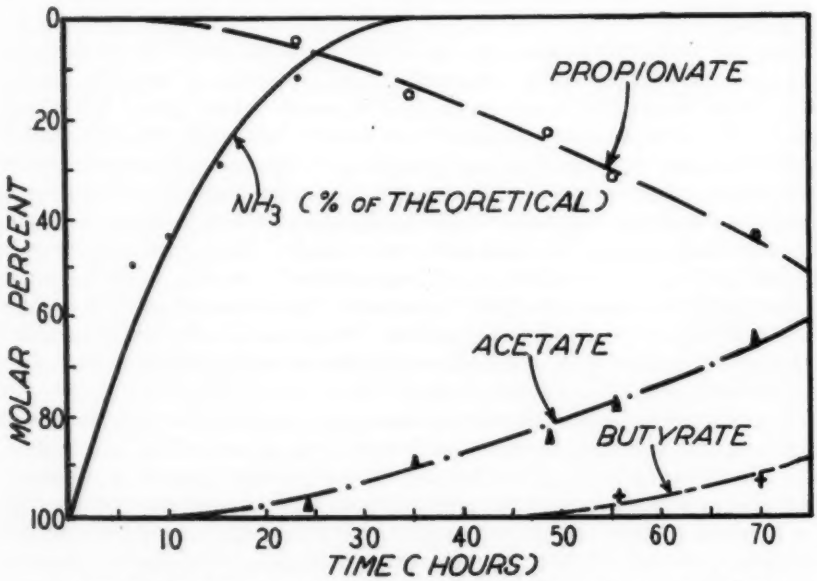

FIG. 2. Fatty acids produced from aspartate at different intervals during a 72-hour incubation.

(approximately 40%) approached that of propionate (55%). The amount of free ammonia present at each interval also is plotted to indicate the rate at which aspartate was dissimilated. These values are calculated on the basis of a theoretical yield of 150 μM (100% dissimilation). The results reported above are based on findings of a series of replicate experiments done over a period of one year.

DISCUSSION

The diagram presented in Figure 3 illustrates some of the more probable pathways in the dissimilation of aspartate to fatty acids.

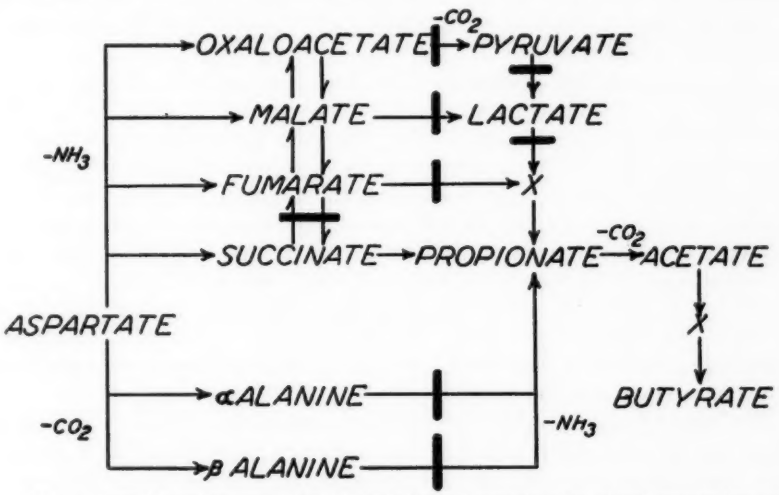

FIG. 3. Possible pathways in the dissimilation of aspartate by mixed suspensions of bovine rumen bacteria.

— Reaction does not conform to data.

The fact that both α- and β-alanine were not attacked by the mixed suspensions would seem to indicate that the degradation of aspartate does not proceed through either of these two compounds. Identical results obtained with cell-free extracts tend to eliminate the possibility of a permeability factor. In addition, the ammonia values obtained during the inhibition experiments (Figure 1), which were higher than corresponding values for carbon dioxide, also indicate that a deamination reaction occurs first. If aspartate was first decarboxylated, the quantity of ammonia produced could not be greater than the quantity of carbon dioxide.

The immediate degradation of aspartate appears to take place by means of a reductive deamination to succinate. Oxidative, hydrolytic, and desaturation deamination to oxaloacetate, malate, and fumarate, respectively, does not seem to occur. Data obtained manometrically and from an analysis of the end products make them inacceptable as intermediates. First, oxaloacetate is not attacked by either intact cells or cell extracts. Second, the amount of carbon

dioxide produced from one molecule of malate was greater than that obtained from aspartate. Lastly, fatty acids were not produced from oxaloacetate, and the types initially produced from malate are different from those produced from aspartate. On the other hand, data derived from succinate are in close agreement with those obtained from aspartate.

The difference in end products obtained from succinate, fumarate, and malate and the fact that no activity occurred with oxaloacetate also would indicate that a scheme involving the interconversion of these four dicarboxylic acids is not present in the rumen. The members of such a scheme should be attacked at similar rates and produce the same end products. Further, the use of 0.01 *M* malonate should affect decarboxylation by inhibiting the dehydrogenation of succinate to fumarate.

If it is assumed that oxaloacetate was decarboxylated to pyruvate and malate to lactate, the data obtained with these C_3 substrates are still incompatible with the data obtained from aspartate. The data presented in this paper are in agreement with results of similar studies presented by Doetsch *et al. (2)*.

The only series of reactions postulated which appear to agree with the data presented includes the direct decarboxylation of succinate to propionate. The inhibition of succinic decarboxylase by 0.3 *M* malonate was previously shown by Delwiche *(3)*. His results are comparable to those obtained here with aspartate as well as with succinate. The direct decarboxylation of succinate was also previously observed by Sypesteyn and Elsden *(10)*, using mixed suspensions of organisms obtained from the sheep rumen. Similar observations made with individual species were reported by Johns *(7)* and Delwiche *(3)*.

The further conversion of propionate to acetate and eventually to butyrate, which was observed experimentally, serves to partially explain the presence of these three fatty acids. Studies on the interconversion of fatty acids in the rumen were made earlier by Gray *et al. (6)*.

The observations presented above are not offered to support any view proposed in the controversy involving the existence of a succinic decarboxylase. An attempt was made to determine the manner in which a single compound is attacked and subsequently degraded by a mixed enzymatically competing bacterial population. It would not be incorrect to assume that any one of the schemes proposed in Figure 3 might be characteristic of a metabolic pattern of a single species present. The conversion of the aspartate which was observed might be the result of the most active portion of many of these individual enzyme systems acting synergistically.

The data presented here allow the following conclusion: The degradation of aspartate by a mixed suspension of rumen bacteria proceeds mainly by a reductive deamination to succinate, followed by a decarboxylation to propionate, which in turn is partially converted to acetate and butyrate.

SUMMARY

The dissimilation of aspartate by mixed suspensions of bovine rumen bacteria was studied in detail. The feasibility of succinate, malate, fumarate, pyruvate,

lactate, α-alanine, and β-alanine as intermediates was considered. Data were obtained manometrically by inhibition experiments and by an analysis of the end products. The principal pathway appears to be a deamination to succinate, decarboxylation to propionate, and further conversion to acetate and butyrate.

REFERENCES

(1) CONWAY, E. J. *Microdiffusion Analysis and Volumetric Error.* 3rd ed. Crosley Lockwood, London. 1950.

(2) DOETSCH, R. N., ROBINSON, R. Q., BROWN, R. E., AND SHAW, J. C. Catabolic Reactions of Mixed Suspensions of Bovine Rumen Bacteria. *J. Dairy Sci.*, **36**: 825. 1953.

(3) DELWICHE, E. A. Mechanism of Propionic Acid Formation by *Propionibacterium pentosaceum*. *J. Bacteriol.*, **56**: 811. 1948.

(4) ELSDEN, S. R. The Application of the Silica Gel Partition Chromatogram to the Estimation of Volatile Fatty Acids. *Biochem. J.*, **40**: 252. 1946.

(5) EL-SHAZLY, K. Degradation of Protein in the Rumen of the Sheep. 2. The Action of Rumen Microorganisms on Amino Acids. *Biochem. J.*, **51**: 647. 1952.

(6) GRAY, F. V., PILGRIM, A. F., RADDA, H. J., AND WHEELER, R. A. Fermentation in the Rumen of the Sheep. IV. The Nature and the Origin of the Volatile Fatty Acids in the Rumen of the Sheep. *J. Exptl. Biol.*, **29**: 57. 1952.

(7) JOHNS, A. T. The Mechanism of Propionic Acid Formation by *Veillonella gazogenes*. *J. Gen. Microbiol.*, **5**: 326. 1951.

(8) LIDDEL, H. F., AND RYDON, H. N. A New Indicator for Use in Partition Chromatography; 3,6-disulpho-β-naphthalene-azo-N-phenyl-α-naphthylamine. *Biochem. J.*, **38**: 68. 1944.

(9) SIROTNAK, F. M., DOETSCH, R. N., BROWN, R. E., AND SHAW, J. C. Nitrogen Metabolism of Bovine Rumen Bacteria. *J. Dairy Sci.*, **36**: 117. 1953.

(10) SYPESTEYN, A. K., AND ELSDEN, S. R. The Metabolism of Succinic Acid in the Rumen of Sheep. *Biochem. J.*, **52**: 41. 1952.

(11) UMBREIT, W. W., BURRIS, R. H., AND STAUFFER, J. F. *Manometric Techniques and Tissue Metabolism.* Burgess Publishing Co., Minneapolis, Minn. 1949.

A COMPARATIVE STUDY OF HUMAN, COW, SOW, AND RAT MILK USING PAPER CHROMATOGRAPHY

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The chromatographic observation of oligosaccharides in lactase hydrolyzed lactose preparations *(1, 13, 15, 17)* has stimulated interest in this laboratory as to the type of sugar components associated with lactose in milks of lactating mammals.

Caputto *et al. (2)*, using benzidine acetate as a spray reagent, detected three spots above lactose in addition to glucose and lactose in extracts of rat mammary glands. Also, very faint traces of two of these spots were detected when they chromatographed human and cow's milk. Honer and Tuckey *(4)* have reported three spots in fresh cow's milk slower moving than lactose, as well as free galactose and glucose. Polonovski and Lespagnol *(14)* have reported the presence of two other disaccharides, allolactose and gynolactose, in human milk. The presence in human milk of nitrogen-containing sugars composed of *N*-acetyl D-glucosamine, L-fucose, and D-galactose has been reported *(3, 6)*. One of the nitrogen-containing oligosaccharides isolated from human milk has been identified as a tetrasaccharide which on hydrolysis yielded one mole each of glucosamine, glucose, and acetic acid and two moles of galactose *(7)*.

We wish to report the initial results obtained when a chromatographic technique for sugars *(11)* was applied to a number of milks.

EXPERIMENTAL

Preparation of milk samples. The samples of fresh, raw mammalian milk used in this study were as follows: human (Caucasian) milk: four samples, 3, 7, 23, and 44 days postpartum; cow's (Holstein) milk: five samples, 3, 27, 51, 75, and 147 hours postpartum; rat's (Sprague-Dawley) milk: four samples, 1, 3, 6, 13 days postpartum; and sow's (Poland-China) milk: one sample, lactating day unknown. The milk samples were taken from one human and from one individual animal of each species.

In preparing the samples for chromatography, equal amounts of the milks in each series were deproteinized with equivalents of approximately 0.3 *N* zinc sulfate and barium hydroxide *(16)*. The deproteinized filtrates were concentrated to dryness under reduced pressure and the sugars were separated from the salts by extracting the residues with anhydrous pyridine *(8)*.

Paper chromatography. Descending one-dimensional chromatograms were made on Schleicher and Schuel No. 589 White Ribbon filter paper with the solvent system ethyl acetate-pyridine-water (2.5:1.0:3.5). The chromatographic

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development was carried out in a temperature-controlled room (22 ± 2° C.). The apparatus and technique for the separation and identification of the sugars have been described previously *(11)*.

In preliminary screening studies it was found that a single 18-24 hour development did not adequately separate the components present in the deproteinized and desalted milk. Therefore, the technique of multiple descent *(5)* was utilized to give the desirable separation of components which had small differences in their R_f values. It was found that two or three 24-hour developments gave satisfactory chromatographic patterns of the components present. The same solvent system and direction of travel was used in each repetition as in the original development.

In the chromatographic procedure used in this study *(11)* the silver nitrate (0.15 *M*, used for detecting the position of the substances on the paper strips) was incorporated directly into the solvent system. A paper strip subjected to repeated irrigations with the solvent system containing the silver nitrate resulted in an overloading of silver nitrate on the paper with a subsequent increase in background discoloration on color development. Therefore, the silver nitrate was incorporated into the solvent system only for the final 24-hour chromatographic development.

The silver nitrate when incorporated directly into the solvent phase became homogeneously distributed throughout the filter paper strip. Exposure of the chromatogram to ammonia vapors after air drying thoroughly saturated the paper strip with ammonia. This procedure permitted the development of spots on exposure to the atmosphere that were not made visible by the 20-minute heat treatment at 80° C. The application of this color development procedure followed by exposure of the chromatogram to the atmosphere for 2 hours permitted the detection of 0.1 γ each of galactose and glucose. This meant that 0.01% of glucose or galactose could be identified in a solution by simply applying 1 μl. of such a solution to a paper strip and utilizing the chromatographic procedure described here. However, since it was an easy matter to apply 100 μl. (50 applications of 2-μl. quantities) to the paper, 0.1 mg. per 100 ml., or 0.0001%, of glucose or galactose could be detected. It has been found that 0.00006% glucose or galactose could have been detected if the ripening time (exposure to the atmosphere) had been extended to 24 hours or more.

RESULTS

Table 1 lists the R_L [1] values of the spots (giving a positive color reaction with ammoniacal silver nitrate) detected in a representative sample of each mammalian milk studied. The samples of human milk taken at 3, 7, and 23 days postpartum were chromatographed on one paper strip for comparison (Figure 1). The sample taken at 44 days postpartum was chromatographed separately. All samples contained 11 spots in addition to lactose and glucose. The maximum

[1] R_L is the ratio of the distance moved by the spot to the distance moved by lactose on the same paper.

TABLE 1

The chromatographic patterns of representative samples of four mammalian milks after the removal of proteins and salts

Source of milk	Lactating days	R_L [a] Values of unknown spots														Galactose	Glucose	Sum [b]
Human	23	0.00	0.04	0.08	0.11	0.19	0.29	0.35	0.42	—	0.68 [c]	0.86	1.20	—	—	No	Yes	13
Cow	4	0.00	—	0.07	0.13	0.24	—	0.36 [c]	0.44	0.53	0.66	0.80 [c]	1.20	—	—	Yes	Yes	13
Sow	Unknown	—	0.05	—	0.15	—	—	0.30	0.44	—	0.62	0.79 [c]	—	1.34	1.55	Yes	Yes	11
Rat	13	—	—	—	—	0.19	—	0.31 [c]	0.42	0.48	—	0.78	1.20	—	—	No	No	7

[a] R_L is the ratio of distance moved by the spot to the distance moved by lactose on the same paper.

[b] The total number of substances, including lactose, galactose, and glucose, which gave a brown colored spot with ammoniacal silver nitrate.

[c] Most prominent unknown spot.

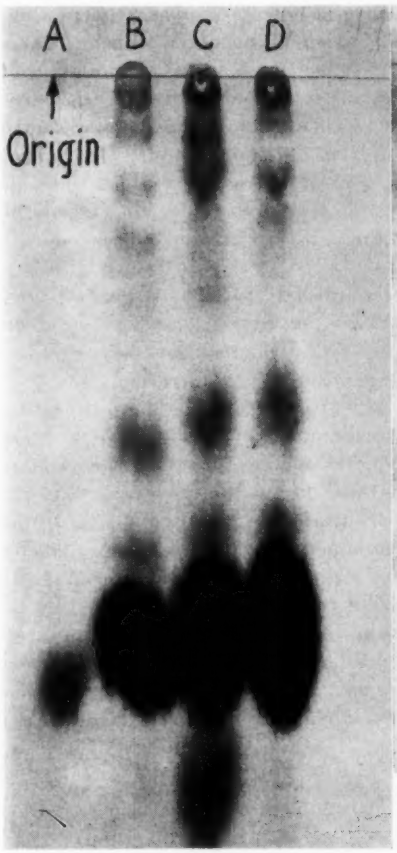

FIG. 1. A one-dimensional descending paper chromatogram of a series of human milk samples after the removal of proteins and salts. That portion of the chromatogram occupied by glucose is not shown.

A—Lactose Standard, B—Human milk, 3 days postpartum, C—7 days postpartum, D—23 days postpartum.

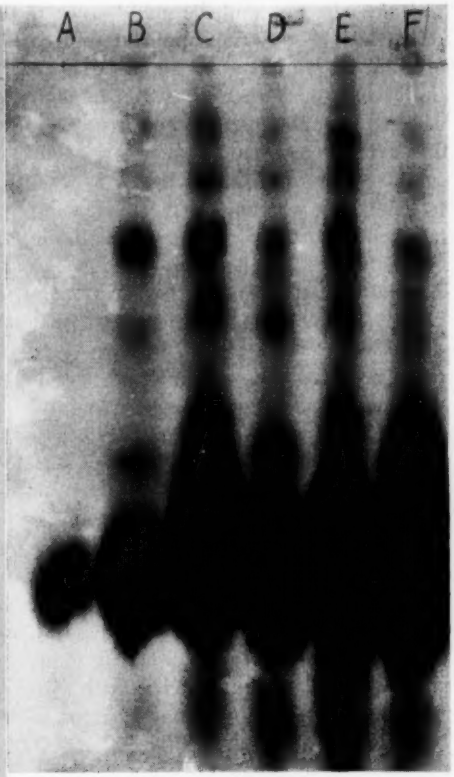

FIG. 2. A one-dimensional descending paper chromatogram of a series of cow's milk samples after the removal of proteins and salts. That portion of the chromatogram occupied by galactose and glucose is not shown.

A—Lactose standard, B—Cow's milk, 3 hours postpartum, C—27 hours postpartum, D—51 hours postpartum, E—75 hours postpartum, F—147 hours postpartum.

concentration of materials detected appeared in the 7-day sample. There was a definite decrease of three spots (R_L = 0.29, 0.35, 0.42) in the 23-day sample, and this decrease was further pronounced in the 44-day sample. The most prominent spot (excluding lactose) in these human milk samples was the second spot (R_L = 0.68) above lactose.

The five samples of cow's milk were all processed identically and chromatographed on one paper strip for comparison. The developed chromatogram showed

that the pattern in all the samples was the same (Figure 2), apparently differing quantitatively but not qualitatively. In addition to lactose and faint traces of galactose and glucose, 10 other spots were detected. A visual examination of the chromatogram (Figure 2) revealed that the lowest concentration of lactose occurred in the 3-hour sample with an increase in each subsequent sample until a maximum concentration was reached in the 147-hour sample. Additional samples were not taken after 147 hours, and it therefore was not possible to determine whether the 147-hour sample represented *(a)* a leveling off of the lactose concentration or *(b)* a maximum value with a subsequent decrease to a constant value as lactation progressed.

The concentration of the additional spots appeared to be at the lowest level in the 3-hour sample, reached a maximum in the 75-hour sample, and then decreased again in the 147-hour sample. The small amount of monoses remained fairly constant without apparent increase or decrease in concentration.

The first three samples of rat's milk (1, 3, 5 days postpartum) were developed on one paper chromatogram for direct comparison. Six spots in addition to lactose were detected. No galactose and glucose were observed. The lactose concentration appeared to be greater in the third-day sample as compared to the first-day. The spot having an R_L value of 0.31 appeared most prominently in the 3-day sample. This spot appeared on the chromatogram almost equal in intensity to that of the lactose spot. A chromatogram of the 13th-day sample (Figure 3) depicts the pattern common to the four samples chromatographed.

The sample of sow's milk contained, in addition to lactose, trace quantities of galactose and glucose, and eight additional spots. Of the eight additional spots detected (Figure 4) the four directly above lactose were the most prominent, with the spot immediately above lactose on the chromatogram ($R_L = 0.79$) being the most pronounced.

DISCUSSION

The photographs of the chromatograms of the milk samples (Figures 1-4) give a qualitative picture of the patterns obtained when subjecting the samples to a procedure for the chromatographic analysis of sugars *(11)*.

A number of the components present in the various milk samples did not become visible on the chromatograms until the paper strips were allowed to "ripen" in the air for several hours. Since the procedure used permitted the detection of as little as 0.0001% of glucose or galactose, it appeared that some of the components detected were present in the milk in quantities of the order of parts per million. Heretofore such components have gone undetected, for the majority of color reagents used in chromatographic analysis are sprayed on the paper strip, and such procedures are not sufficiently sensitive to detect such trace quantities of material.

Comparing the chromatographic patterns for the four milks, the most striking observation was the difference in the color intensity of each of the spots present, their positions on the chromatogram, and the differences in the position of the main spot in each sample. Human milk consistently showed a series of four spots

FIG. 3. A one-dimensional descending paper chromatogram of a sample of rat's milk, 13 days postpartum, after the removal of proteins and salts. "A" is the main unknown substance (R_L = 0.31) found to be composed of galactose and glucose, and "B" is lactose.

FIG. 4. A one-dimensional paper chromatogram of a sample of sow's milk after the removal of proteins and salts. A is lactose.

FIG. 5. A one-dimensional descending paper chromatogram showing:

A—Lactose standard.
B—The purified substance (R_L = 0.31) obtained from rat's milk after the removal of proteins and salts.

near the origin with one located directly at the origin, whereas this series was not present in the samples of cow, sow, and rat milk.

Based simply on a visual comparison of spot intensities, a natural conclusion drawn was that the main additional spot (R_L = 0.31) found in rat milk closely approximated the concentration of the lactose present. This has not been sub-

stantiated quantitatively, but mention is made of its relative concentration in order to point out its prominence in rat milk.

It was found that in addition to the small quantities of fresh rat milk available, the stomach contents of 3-day-old suckling rats which were sacrificed provided a rich source of this substance. Except for the addition of galactose and glucose in the milk obtained from the stomachs *(10)*, the chromatographic patterns of the two sources of rat milk were identical. Using the stomach milk as a source of this substance, it was eluted off a paper chromatogram (Figure 5) and then acid hydrolyzed *(12)*. A chromatogram of the clarified hydrolyzate revealed that it was composed of galactose and glucose. The chromatogram also revealed traces of lactose, indicating that lactose was a component part of the substance. However, the fact that the substance may be a disaccharide and simply an isomeric form of lactose cannot be discounted at this time. The presence of lactose may be the result of a transgalactosidation reaction (glucose serving as the acceptor) initiated by the hydrolyzing acid *(1, 9, 15)*.

Aside from this single isolation, no attempt has been made to isolate the additional components detected in each milk in order to determine whether they are carbohydrates. The preparation of the milk samples prior to chromatographic assay, the positions the additional substances occupied on the chromatogram, and the presence of other carbohydrates in milks as reported by various investigators *(2, 3, 4, 6, 7, 10, 14)* are strong indications that the additional spots represent substances of a carbohydrate nature. However, only their characterization following their isolation will serve to establish their true identity. If they are carbohydrates, the differences in the chromatographic patterns of each milk may signify that the additional substances may have some function in the nutritional requirements of the respective animal.

SUMMARY

The analyses of human, cow, rat, and sow milk after the removal of proteins and salts have disclosed some marked differences in their respective chromatographic patterns. All, of course, contained lactose. In addition, human milk contained 11 other unidentified spots as compared to eight for sow's milk, six for rat's milk, and ten for cow's milk. Glucose was found in human milk and galactose and glucose in cow's and sow's milk, whereas rat's milk contained neither galactose nor glucose.

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REFERENCES

(1) Aronson, M. Transgalactosidation During Lactose Hydrolysis. *Arch. Biochem. Biophys.*, **39**: 370. 1952.

(2) Caputto, R., and Trucco, R. E. A New Galactose-Containing Compound from Mammary Glands. *Nature*, **169**: 1061. 1952.

(3) György, P. A Hitherto Unrecognized Biochemical Difference between Human Milk and Cow's Milk. *Pediatrics*, **11**: 98. 1953.

(4) Honer, C. J., and Tuckey, S. L. Chromotographic Studies of Reducing Sugars, Other than Lactose, in Raw and Autoclaved Milk. (Abs., A.D.S.A. 49th Ann. Meeting) *J. Dairy Sci.*, **36**: 559. 1953.

(5) Jeanes, A., Wise, C. S., and Dimler, R. J. Improved Techniques in Paper Chromatography of Carbohydrates. *Anal. Chem.*, **23**: 415. 1951.

(6) Kuhn, R. Vitamine der Milch. *Angew. Chem.*, **64**: 493. 1952.

(7) Kuhn, R., Gauhe, A., and Baer, H. H. Über ein N-haltiges Tetrasaccharid aus Frauenmilch. *Chem. Ber.*, **86**: 827. 1953.

(8) Malpress, F. H., and Morrison, A. B. Use of Pyridine in the De-ionization of Solutions for Paper Chromatography. *Nature*, **164**: 963. 1949.

(9) Malyoth, G., and Stein, H. W. Beitrag zur Spaltung der Lactose. *Angew. Chem.*, **64**: 399. 1952.

(10) Malyoth, G., Stein, H. W., Hörmann, E., and Schuler, R. Papierchromatographische Untersuchungen von Frauenmilch, Kuhmilch and Rattenmilch. *Experientia*, **9**: 70. 1953.

(11) McFarren, E. F., Brand, K., and Rutkowski, H. R. Quantitative Determination of Sugars on Filter Paper Chromatograms by Direct Photometry. *Anal. Chem.*, **23**: 1146. 1951.

(12) Partridge, S. M. Filter Paper Partition Chromatography of Sugars. *Biochem. J.*, **42**: 251. 1948.

(13) Pazur, J. H. The Enzymatic Conversion of Lactose into Galactosyl Oligosaccharides. *Science*, **117**: 355. 1953.

(14) Polonovski, M., and Lespagnol, A. Sur deux nouveaux sucres du lait de femme, le gynolactose et l'allolactose. *Compt. rend.*, **192**: 1319. 1931.

(15) Roberts, H. R., and McFarren, E. F. The Chromatographic Observation of Oligosaccharides Formed During the Lactase Hydrolysis of Lactose. *J. Dairy Sci.*, **36**: 620. 1953.

(16) Somogyi, M. Determination of Blood Sugar. *J. Biol. Chem.*, **160**: 69. 1945.

(17) Wallenfels, K. Enzymatische Synthese von Oligosacchariden aus Disacchariden. *Naturwiss.*, **38**: 306. 1951.

A COMPARISON OF SOME CELLULAR AND CHEMICAL CONSTITUENTS OF BLOOD AT TIME OF PARTURITION AND AFTER ADMINISTRATION OF ADRENOCORTICOTROPHIN[1]

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Undoubtedly, numerous physiological mechanisms are concerned in an attempt to maintain homeostasis immediately prior and subsequent to paturition in the cow. Evidence for the failure of these mechanisms are the many complications associated with parturition and accompanying initiation of lactation. The role of various buffer systems in meeting stress conditions has not been extensively studied in dairy cows.

The adrenal cortex has been assigned a role of major importance in increasing the resistance of the organism to stress *(11, 13)*. In normal animals there is a marked diminution in the number of circulating eosinophils in response to the injection of adrenocorticotrophic hormone (ACTH), epinephrine, and insulin and to the stimuli of various stresses *(11, 13)*. Since Hills *et al. (9)* reported that the number of circulating eosinophils is indicative of increased adrenal cortical secretion, the eosinophil response test has been widely used as an assay of adrenocortical function *(11, 12, 13)*. However, Thorn *et al. (13)* have shown that eosinopenia of stress cannot be definitely attributed to an increased secretion of the adrenal cortex unless there is a concomitant increase in urinary corticoids.

A lymphopenia also occurs as a result of increased adrenocortical activity *(3, 4)*. A lymphopenia has been used as an index of adrenocortical activity, but the drop in number of circulating lymphocytes in man is not as great as the eosinophils and the response is more variable *(7)*. However, Sayers *(11)* is of the opinion that the number of circulating lymphocytes is a valuable index of adrenocortical activity in those species in which the lymphocytes make up the greatest percentage of the white blood cells or leucocytes as in the bovine. In addition, Kerr *et al. (10)* state that the change in the ratio of lymphocytes to neutrophils is a more reliable index of adrenocortical function in cattle than an eosinopenia.

An eosinopenia has been observed during the stress of labor in women and for the first day postpartum *(2)*. In cattle, Ferguson and coworkers *(6)* noted a general decrease in the per cent of neutrophils and a concomitant increase in the lymphocytes during the 10-day post-calving interval. There was also a significant decrease of the total number of leucocytes for the same period, as compared to the precalving level. In a study of *Trichomonas fetus* infection in cattle, Kerr *et al. (2)* observed a loss of skin sensitivity to the *T. fetus* antigen

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and a lymphopenia at time of parturition. Similar reactions had been obtained by injections of cortisone.

This study was undertaken to investigate the effect of parturition on changes in the leucocytes and blood sugar as compared to injections of ACTH.

EXPERIMENTAL PROCEDURE

Studies on parturition. Cows of the Holstein, Guernsey, Brown Swiss, and Jersey breeds in the University herd were used in this study. Blood samples were drawn from the jugular vein daily beginning 5 days before the anticipated day of calving. An attempt was made to obtain blood samples at more than and less than 6 hours before calving. Many of the samples in the less than 6-hour category were drawn within a few minutes before calving. Samples were also drawn at similar intervals subsequent to calving. The anticoagulant used was a mixture of 40% potassium oxalate and 60% ammonium oxalate, which causes a minimum change in cell volume *(8)*. Blood was collected in 15-ml. centrifuge tubes in which 1 ml. of a 2% solution of the anticoagulant had been evaporated to dryness. Total leucocytes were counted in a hemacytometer with Neubauer rulings. Two smears were made from each sample of blood and were stained with Wright's stain. One hundred cells from each slide were counted for the differential counts.

Studies with ACTH. Holsteins, Guernseys, and Jerseys were used to study the effect of the administration of ACTH. Daily blood samples were drawn 3 days prior to the injection of ACTH, at 8, 12, 16, and 24 hours after injection, and daily for three consecutive days thereafter. The 250 I.U. of ACTH were administered intramuscularly. ACTH was used for comparative purposes instead of cortisone because it was believed a more complete reaction would be obtained by the use of ACTH.

A Folin-Wu *(5)* protein-free blood filtrate was used to determine the blood sugar by the Benedict method *(1)*.

RESULTS

Effect of parturition. The results of the effect of parturition on the reaction of leucocytes and blood sugar are presented in Table 1. Only cows calving normally were included. The values obtained from some cows were eliminated from the postpartum data because of retained placentas, which resulted in a tremendous increase in the numbers of lymphocytes. The leucocytes attained peak concentration at the 5:59 to 0:00 prepartum interval. Previous to that time, the leucocyte concentration was relatively constant. A decline in leucocytes occurred at each interval from the peak of 11,900 to 6,600 on the fifth day postpartum. The leucocyte concentration for the postpartum interval was considerably below the prepartum level.

On the day of parturition there was a marked decrease in the per cent of lymphocytes. The per cent of lymphocytes increased during the postpartum interval to a level higher than that which existed during the prepartum interval studied. The mean change in the concentration of lymphocytes caused a relative

TABLE 1

Effect of parturition on W.B.C. and blood sugar

	Days prepartum					Day of parturition (hours)				Days postpartum				
	5	4	3	2	1	6:00 plus	5:59 0:00	0:00 5:59	6:00 plus	1	2	3	4	5
	(14)[a]	(16)	(17)	(21)	(22)	(10)	(20)	(25)	(17)	(21)	(16)	(16)	(16)	(12)
Leucocytes per cmm.	9,200	9,200	8,900	9,700	10,100	9,100	11,900	11,000	10,100	8,200	7,600	7,000	6,800	6,600
						Cells per 100 leucocytes								
Lymphocytes	56.8	54.2	55.3	55.6	51.9	46.1	47.9	44.4	45.4	54.5	58.3	61.2	65.4	62.8
Neutrophils	31.3	33.8	32.9	33.2	37.2	46.3	44.4	49.9	47.8	36.4	30.7	27.0	24.0	25.6
Eosinophils	9.3	9.0	9.0	8.8	8.4	5.3	4.7	3.3	3.2	6.1	8.4	7.4	7.2	6.9
Monocytes	4.6	4.3	4.2	4.1	4.2	3.5	4.2	3.7	4.4	4.7	3.7	4.9	4.6	5.2
	(10)[a]	(10)	(12)	(15)	(15)	(8)	(13)	(18)	(16)	(17)	(18)	(18)	(17)	(14)
Blood sugar *(mg. %)*	43.3	41.9	42.9	43.6	48.3	50.1	57.9	65.2	55.4	51.0	43.7	44.8	43.0	41.3

[a] Figures in parenthesis indicate the number of cows included in the mean of each category

lymphopenia (decrease in the per cent of lymphocytes), but not an absolute lymphopenia (decrease in the total number of lymphocytes). However, the mean values for the blood constituents (Table 1) tend to mask the magnitude and period of response of individual cows, since maximum response occurred at varying times in relation to parturition. When the total number of the different types of cells was calculated for individual cows, 17 cows had an absolute lymphopenia, four cows had only a relative lymphopenia, and four cows showed changes of insufficient magnitude that there was no crossing over in the percentage of lymphocytes and neutrophils. The time of maximum response as indicated by a maximum eosinopenia and a cross-over between the lymphocytes and neutrophils for individual cows is as follows: three cows at 6 hours or more prepartum, 12 cows from 6 hours prepartum to 6 hours postpartum, and six cows at 6 hours or more postpartum. Thus the interval from parturition to the time when a cow exhibits a maximum response to the stress of parturition varies. The mean values show, as might be expected, that the greatest response to stress occurs the day of parturition.

An increase in neutrophils commensurate with the decrease in lymphocytes occurred the day of parturition. In contrast to the lymphocytes, the concentration of neutrophils postpartum was below the prepartum level. The differential count of the leucocytes included a division of the neutrophils into segments (mature neutrophils) and stabs (immature neutrophils). The stabs represented a larger percentage of the total neutrophils immediately prior to and during the period of maximum response to stress, as indicated by the change in types of cells. The increase in immature neutrophils would be expected with an increase in total leucocytes, a lymphopenia, and an eosinopenia. However, in reporting the results, the segments and stabs are grouped together.

The eosinophils were reduced in each of the intervals on the day of parturition and had not attained the prepartum level by the fifth day postpartum. The maximum eosinopenia occurred during the same intervals as the maximum lymphopenia.

These results indicate no observable effects on the monocytes. Although the basophils were counted, the concentration was erratic and did not present a consistent pattern, and no attempt was made to include them in a mean.

The blood sugar level began increasing the first day prepartum and reached a high of 65.2 mg. per cent in the 0:00 to 5:59 postpartum interval. By the second day postpartum the blood sugar was down to the prepartum level.

Effect of ACTH. The total leucocytes were fairly constant for the preinjection days of the study on ACTH (Table 2). At 8 hours after injection, the total leucocytes had increased, and they reached a peak at 16 hours. On the second postinjection day, the total leucocytes were back to the preinjection level.

Eight hours postinjection a marked diminution of the lymphocytes occurred, which persisted through the 16-hour interval. The per cent of lymphocytes increased to the preinjection level by the second day postinjection. The time that individual cows showed a maximum response after injection of ACTH was as follows: three at 8 hours, three at 16 hours, and one at 24 hours. Because of the

TABLE 2

Effect of ACTH on W.B.C. and blood sugar

	Days preinjection			Hours postinjection				Days postinjection		
	3	2	1	0	8	16	24	2	3	4
Leucocytes per cmm.	8,900	8,800	8,900	8,900	13,000	13,400	9,800	9,600	8,700	8,000
				Cells per 100 leucocytes						
Lymphocytes	58.2	60.2	63.1	66.9	39.6	42.2	47.8	60.4	58.9	58.9
Neutrophils	24.9	21.5	23.8	22.0	55.0	53.8	46.1	24.3	21.2	24.9
Eosinophils	14.4	10.1	10.2	9.2	3.1	2.1	3.9	15.2	18.1	16.0
Monocytes	3.3	3.0	3.4	2.3	3.1	2.5	2.9	3.8	3.8	4.0
Blood sugar *(mg. %)*	46.4	47.4	52.1	53.7	63.8	78.9	74.3	52.9	51.2	48.1

variation in time of response, the mean values show a relative lymphopenia, but on an individual basis all seven cows showed an absolute lymphopenia.

The injection of ACTH caused a 50% increase in the per cent of neutrophils 8 hours after injection. The neutrophils still were considerably above preinjection levels 24 hours postinjection but had decreased to normal levels the second day postinjection.

The eosinophils responded to the administration of ACTH with a marked decrease in concentration occurring 8 hours postinjection and persisting through the 24-hour interval. The per cent of eosinophils was higher the third and fourth days postinjection than during the preinjection days.

There was much variation in the per cent of monocytes, both prior and subsequent to injection. ACTH appeared to have no effect on the monocytes.

The blood sugar had increased 20 mg. per cent above the preinjection level at 16 hours after injection. The level had returned to normal by the second day after injection.

DISCUSSION

There are marked similarities between the reaction of the leucocytes and blood sugar to parturition and to the administration of 250 I.U. of ACTH. Both stimuli caused an increase in the total leucocytes, but the response to the injection of ACTH was greater. A dissimilar reaction was the decrease of the total leucocytes postpartum below the prepartum level, whereas with ACTH the total leucocytes had returned to the preinjection level by the second day postinjection.

The reaction of the lymphocytes, neutrophils, and eosinophils to parturition and ACTH administration was all in the same direction, but the magnitude of change was greater with the ACTH. The eosinophils behaved differently after the administration of ACTH than after parturition, in that they exceeded the preinjection level on days 2, 3, and 4 postinjection.

An increment in blood sugar occurred during parturition and after administration of ACTH, but the increment was greater and was sustained longer with ACTH.

These results indicate a greater degree of stimulation from the dose of ACTH administered in these studies than from the stress of parturition.

SUMMARY

A comparison was made of the reaction of leucocytes and blood sugar to parturition and to the administration of 250 I.U. of ACTH. The stress of parturition caused a diminution in the per cent of circulating lymphocytes and eosinophils and an increment in the neutrophils and blood sugar. Administration of ACTH also caused a diminution in the per cent of circulating lymphocytes and eosinophils and an increment in the neutrophils and blood sugar. Generally the magnitude of change was greater from the dose of ACTH administered than from the stimulus of parturition.

The results of this study indicate an increased secretion from the adrenal cortex in response to the stress of parturition in the cow.

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REFERENCES

(1) Benedict, S. R. The Analysis of Whole Blood. II. The Determination of Sugar and of Saccharoids (Non-Fermentable Copper-Reducing Substances). *J. Biol. Chem.*, **92**: 141. 1931.

(2) Davis, M. E., and Hulet, B. E. Changes in Circulating Eosinophils in Women During the Menstrual Cycle and Reproduction. *J. Clin. Endocrinol.*, **9**: 714. 1949.

(3) Dougherty, T. F., and White, A. Influence of Hormones on Lymphoid Tissue Structure and Function. The Role of the Pituitary Adrenotrophic Hormone in the Regulation of Lymphocytes and Other Cellular Elements of the Blood. *Endocrinol.*, **35**: 1. 1944.

(4) Dougherty, T. F., and White, A. An Evaluation of Alterations Produced in Lymphoid Tissue by Pituitary-Adrenal Cortical Secretion. *J. Lab. Clin. Med.*, **32**: 584. 1947.

(5) Folin, O., and Wu, H. A System of Blood Analysis. *J. Biol. Chem.*, **38**: 81. 1919.

(6) Ferguson, L. C., Irwin, M. R., and Beach, B. A. The Effect of Parturition on the Blood Picture of Cows in Health and During Infection with *Brucella Abortus*. *Am. J. Vet. Research*, **2**: 394. 1941.

(7) Forsham, P. H., Thorn, G. W., Prunty, F. T. G., and Hills, A. G. Clinical Studies with Pituitary Adrenocorticotropin. *J. Clin. Endocrinol.*, **8**: 15. 1948.

(8) Heller, V. G., and Paul, H. Changes in Cell Volume Produced by Varying Concentrations of Different Anticoagulants. *J. Lab. Clin. Med.*, **19**: 777. 1934.

(9) Hills, A. G., Forsham, P. H., and Finch, C. A. Changes in Circulating Leucocytes Induced by Administration of Pituitary Adrenocorticotrophic Hormone (ACTH) in Man. *Blood*, **3**: 755. 1948.

(10) Kerr, W. R., McGirr, J. L., and Robertson, M. Specific and Non-specific Desensitization of the Skin in Trichomonas Sensitive Bovines. *J. Comp. Path. Therap.*, **59**: 133. 1949.

(11) Sayers, G. Adrenal Cortex and Homeostasis. *Physiol. Revs.*, **30**: 241. 1950.

(12) Spiers, R. S., and Meyer, R. K. A Method of Assaying Adrenal Cortical Hormones Based on a Decrease in the Circulating Eosinophil Cells of Adrenalectomized Mice. *Endocrinol.*, **48**: 316. 1951.

(13) Thorn, G. W., Jenkins, D., and Laidlow, J. C. The Adrenal Response to Stress in Man. *Recent Progr. Hormone Research*, **8**: 171. 1953. Academic Press, New York.

A SIMPLE TEST OF QUALITY IN HAY [1]

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In an earlier paper *(1)* on the subject of hay quality it was stated that a search for a more readily determined index of roughage quality would be continued. Since then, a simple test based on turbidity of a water extract of hay has been developed which shows considerable promise. The method is a modification of one originated in this laboratory for determining the relative maturity of sweet corn *(2)*.

EXPERIMENTAL

The test is carried out as follows: Three grams of dry hay ground to pass a ½ mm. sieve are suspended in approximately 200 ml. of distilled water containing 4 to 5 drops of concentrated acetic acid. The mixture is agitated for 5 minutes in a Waring blendor, then transferred to a 500-ml. volumetric flask, and made up to volume. The resulting suspension is centrifuged at 1500 r.p.m. for 5 minutes, and any floating particles are removed by pouring the supernatant

TABLE 1

Turbidity of water extract of various hays as related to their chemical composition (all results except the last column are expressed as percentages on the dry matter basis)

Lot No.	Crude protein	Ether extract	Crude fiber	N-free extract	Total ash	Lignin	Total [a] sugar	Turbidity [b]
1	7.6	2.2	35.1	49.9	5.1	6.2	5.9	.231
2	7.8	2.9	35.3	48.6	5.4	5.4	7.0	.233
3	13.3	3.2	32.7	43.5	7.3	7.6	5.0	.295
4	9.3	1.9	37.5	44.6	6.7	5.6	5.1	.262
5	11.3	1.6	36.0	46.1	5.0	5.7	2.5	.324
6	15.4	2.1	32.4	42.2	7.9	6.3	4.3	.374
7	11.4	1.7	34.4	46.2	6.3	5.6	2.3	.326
8	8.4	3.3	37.3	44.2	6.8	7.3	6.8	.274
9	13.2	3.4	33.1	41.5	8.8	9.0	7.3	.315
10	8.7	3.5	39.9	40.3	7.6	7.2	4.5	.256
11	13.7	3.9	33.8	41.5	7.1	5.5	5.5	.241
12	8.5	2.1	38.3	45.8	5.3	7.8	6.3	.192
13	10.0	2.5	37.3	44.5	5.6	8.5	5.9	.267
14	13.4	3.2	31.8	41.8	9.8	10.2	3.4	.359
15	17.5	4.5	24.2	46.1	7.7	8.8	6.3	.370
16	19.2	4.9	21.3	45.8	8.8	8.4	10.0	.473

[a] Expressed as invert sugar.
[b] Expressed as optical density on the galvanometer scale of the spectrophotometer.

liquid through a thin layer of glass wool. Turbidity of the filtered extract is determined in a Coleman spectrophotometer (Model 11) at 675 mμ with a No. PC-5 Corning filter and distilled water as the reference liquid. Results of the test on 16 samples of hay of widely varying proximate composition are given in Table 1.

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Lots 1 to 7 were hays that had been in storage at least several months when sampled; lots 8 to 10 were sampled at the time of storage; lots 11 to 16, strictly speaking, were not hays but oven-dried fresh grasses or legumes, or both, included for purposes of comparison.

DISCUSSION

Preliminary examination of these results indicated some interesting relationships between degree of turbidity of the water extracts and the amounts of certain constituents, notably protein and fiber, as determined in conventional fodder analysis. These relationships have been evaluated statistically, and the values for r appear in Table 2.

The highlights of the r values are the close positive correlation between protein and turbidity in the hays and the almost equally close negative correlation between fiber and turbidity. Total ash also is closely correlated with turbidity;

TABLE 2

Correlation between turbidity and various constituents

Turbidity[a] *of*	r[b]	Significance[c]
Crude protein	0.86	Very high
Ether extract	0.43	Not significant
Crude fiber	−0.82	Very high
N-free extract	−0.15	Not significant
Total ash	0.65	High
Lignin	0.39	Not significant
Total sugar	0.14	Not significant
Protein-fiber ratio	−0.81	Very high

[a] Optical density.

[b] r was determined by means of the formula: $r = \frac{\Sigma xy}{\sqrt{(\Sigma x^2)(\Sigma y^2)}}$

in which x represents the variation from the mean of a single item in the "dependent variable" group of values, and y the same variation in the "independent variable" group, turbidity being classed as the "dependent variable."

[c] Significance has been determined by reference to the table of values for this purpose given by Snedecor *(3)*, using 15 degrees of freedom.

this is not surprising, since high protein and high ash usually are associated in roughages and both of them frequently are associated with low fiber. The negative correlation of turbidity with fiber may be due to the fact that cellulose per se is not readily dispersed to a stable particle size by the means used and/or that the zeta potential is favorable for flocculation. The attractive forces among the particles cause aggregation in a relatively short time. Similar experiments conducted with a high grade cellulose have yielded essentially clear supernates even after repeated treatment in the blendor.

The very low value for turbidity *of* sugar is readily comprehended; all mono- and disaccharides, at least, give clear water solutions. Less clear-cut and harder to understand is the r value for lignin; one might expect it to be similar to that for fiber. The disparity may be due to the fact that lignin per se is more easily dispersed than cellulose, and conditions seem to be more favorable for the production of a suspension that is stable to the amount of centrifugal force employed in the test. Experiments with isolated lignin indicate that it might con-

tribute appreciably to the total turbidity. However, when one considers the particle size and the intimate association of lignin and other cell-wall components in natural material in relation to the dispersive powers used, one might conclude that the possibility of lignin making a significant contribution to turbidity is rather remote.

As might be expected, the value for r between protein-fiber ratio and turbidity is in close agreement with that between fiber and turbidity. Since high protein and high fiber are sometimes associated in roughages, especially in coarse-stemmed legumes, it is thought that the protein-fiber ratio is a better index of feeding value in a roughage than either of these constituents considered singly. This possibility may be of value wherever protein and fiber content already have been determined; otherwise the turbidity test proposed here is much simpler and easier.

Our observations with the turbidity test have been supplemented by carefully controlled feeding trials for milk production with six Holstein cows, using four of the lots of hay (No. 2, 3, 5, and 6). Results of these trials are given in Table 3; for ease of comparison, the protein, fiber, and turbidity values of these lots are repeated there.

The number of trials was too small to warrant statistical evaluation of the correlation between turbidity of the hay extract and milk yield, but an examination of Table 3 indicates that without exception relatively high protein, rela-

TABLE 3

Comparison between turbidity of hay extract and milk yield of cows fed the several lots

Lot No.	Crude protein	Crude fiber	Turbidity[a]	Daily milk yield per cow[b]	Grade of hay[c]
	(%)	*(%)*	*(%)*	*(lb.)*	
2	7.8	35.3	.233	32.1	Poor
3	13.3	32.7	.295	35.4	Good
5	11.3	36.0	.324	26.2	Poor
6	15.4	32.4	.374	35.3	Good

[a] Optical density.

[b] Average for 6 cows for 60 days, *i.e.*: a total of 360 cow-days.

[c] As determined by superficial examination before the feeding trials were started. Lots 2 and 3 were fed during the winter of 1951-52; Lots 5 and 6 during the winter of 1952-53.

tively low fiber, grade of hay as predetermined, and turbidity of the hay extract all bore a close relationship to the amount of milk produced. In the first season (1951-52) a decrease in optical density of the hay extract of 0.062 was accompanied by an average drop in milk yield of 3.3 lb. per cow daily; in the second season (1952-53) a decrease of 0.050 was accompanied by an average drop of 9.1 lb. of milk per cow daily.

It should be noted that these declines in milk production when the "poor" hay was fed cannot be due, except in a very minor degree, to decline in production with advancing lactation. All of the cows in both years were alternated

twice between "good" and "poor" hay, each feeding period being 4 weeks in length. The order of feeding of the hays was as follows:

Cow 1 (each year): good—poor—good—poor
Cow 2 (each year): poor—good—poor—good
Cow 3 (each year): poor—good—poor—good

Thus in two-thirds of the periods (16 out of 24) good hay was fed following poor hay, and in the other third it followed poor hay in two out of eight periods. Also, different cows were used each year.

SUMMARY

A simple test for quality in hay is described, which is based on the degree of turbidity in a water extract of the hay. The test has been shown to be positively correlated to a highly significant degree with high protein and ash content and low fiber content in hay and has been further confirmed by feeding trials for milk produciton with several of the lots of hay tested.

REFERENCES

(1) Archibald, J. G., Bart, J., Blaisdell, M. L., and Spelman, A. F. Quality in Roughages. I. Factors which Influence Hay Composition and Quality. *J. Dairy Sci.*, **34**: 656. 1951.
(2) Johnson, W. B., and Bennett, Emmett. A Turbidity Test for Measuring the Maturity of Sweet Corn. *Proc. Am. Soc. Hort. Sci.*, **59**: 400. 1952.
(3) Snedecor, G. W. *Statistical Methods.* p. 133. Iowa State College Press, Ames. 1938.

NEW ADVANCES IN THE ELECTROEJACULATION OF THE BULL[1]

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It is now generally accepted throughout the dairy industry that the use of a desirably proven sire is one of the most effective methods of improving dairy cattle. Proven bulls by necessity are older bulls and, when considering this group, one of the ailments encountered is the lack of desire on the part of the bull to serve. This is often due to pathological conditions of the feet and joints rather than to the failure of the genital organs. Many bulls of proven value are slaughtered each year because of arthritis or other ailments which make it difficult or impossible for the bull to mount the "teaser cow." There has long been a need for the development of some method which would enable routine collection of semen to be made from bulls which are either unwilling or unable to serve naturally. Much valuable time is often lost waiting for so-called slow bulls to serve, and any practical method for collection which would enable routine ejaculates to be obtained quickly from these animals would be of value to the cattle industry.

The use of an electromotive force for obtaining semen from domestic animals is not new. Gunn *(3)* reports the first successful electroejaculation of a domestic animal. This was obtained from the ram by the insertion of a single pencil-sized metallic electrode into the rectum while a second electrode in the form of a stout needle was embedded in the animal's back. The electromotive force applied was from 10 to 40 v., which was obtained from a small transformer connected to the household power source (alternating current). Although this method caused the emission of semen in the ram, it was not obtained without considerable physical stress to the animal, which was tied on its side during the experiment.

Thibault *et al. (6)* and Laplaud *et al. (5)* obtained electroejaculation from the bull by using a multi-polar electrode consisting of 30 metallic rings separated from each other by ebonite spacers. This electrode was inserted into the rectum, and an electromotive force up to 30 v. at 700 ma. alternating current was applied.

Though electroejaculation has been demonstrated experimentally, its use has received little attention in the field of artificial insemination. This is probably due to the rather severe stress placed on the animal by previously employed techniques and to the fact that ejaculation usually occurs without erection of the penis.

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It was with the hope of developing a more practical method of electroejaculation, which could be employed routinely for the collection of semen for artificial insemination, that our investigation was undertaken.

RESULTS

Our experiments were performed chiefly on two young Hereford bulls. These were ejaculated electrically two or three times weekly for a period of 12 months. Normal ejaculates were obtained throughout the period with the bulls also being used for natural service from time to time. The semen obtained was of good quality, and there was no lack of desire on the part of the bulls to return to natural service. As progress was made in the development of the apparatus, tests were performed in the field on a wide variety of bulls, of ages varying from 2 to 17 years. Both beef and dairy breeds were included in these experiments.

At the outset, a multi-polar electrode consisting of ten 3½-in. diameter copper rings separated by 2-in. plastic disks was employed as an electrode. This was inserted into the rectum of the bull. The electrical stimulation was then applied for 3 to 8 seconds followed by a resting period of about equal length. The power source consisted of a transformer with a variable secondary winding enabling 0 to 130 v. to be obtained. This, in turn, actuated a 20-v. step-down transformer, thus enabling a gradually rising and falling potential to be applied to the electrode. Though this apparatus would cause ejaculation to occur, it was accomplished with stimulation of the lumbar plexus, which caused contraction of the leg and back muscles, as well as considerable discomfort to the animal. It quickly became apparent that the apparatus caused a generalized stimulation of motor and sensory nerves of the lumbar and sacral plexuses, of which stimulation of the ejaculatory center, presumably via the pudendal nerve, was merely incidental. Another difficulty was that though there was little trouble in obtaining semen, ejaculation usually occurred without erection of the penis. The semen trickled out through the preputial sheath, thus becoming contaminated with dirt and bacteria.

It became apparent that before electroejaculation could become a routine practice for semen collection it was necessary to develop a technique which would stimulate the ejaculatory center with minimum stimulation of the sciatic and lumbar nerves. It should be recalled that the impulses which elicit ejaculation *(2)* are parasympathetic and travel over the pudendal nerve. It is also generally considered that an ejaculatory center in the lumbosacral spinal cord integrates the afferent and efferent impulses which cause the emission of semen.

Attention was therefore placed on electrode design. A number of different multipolar electrodes were tried in an endeavor to reduce the amount of power needed to attain ejaculation. Best results have been obtained by the use of a cylindrical lucite electrode 1⅞ in. in diameter and 13 in. long. Inlaid into this cylinder 90 degrees apart are four brass electrodes 11 in. long and 5/16 in. wide. These electrodes are connected in pairs 180 degrees apart (Figure 1).

It was found that if the entire electrode was placed in the rectum so that

only the electric cable remained outside, the discomfort felt by the bull was significantly reduced. There are many nerve endings around the anal sphincter, but by placing the electrode entirely in the rectum pain from this source is eliminated. Although advances in electrode design caused a decrease both in discomfort to the bull and in the total power required for electroejaculation, the technique still left much to be desired.

The investigation then turned to the electric pulse supplied to the electrode. It was soon found that wave form and frequency of the stimulating pulse were most critical. Spike waves ranging from 0.01 to 30 milliseconds duration would cause neither erection nor ejaculation regardless of the frequency employed or the power used, though they readily caused violent muscular contractions of the sciatic and lumbar regions. The use of a pure sine wave pulse was found to give the best results. It is of interest that if the top of the sine wave was removed to form a square wave, the power could be tripled without causing either erection or ejaculation. By merely replacing the top of the pulse to form the original sine wave again, erection and ejaculation readily occurred. The frequency of the stimulating pulse is most critical for best results. Though ejaculation can be induced by the use of a pure sine wave over a fairly wide range of frequency (12 to more than 70 cycles), the best results are obtained in a fairly narrow band. By far the most satisfactory results are obtained by the use of frequencies between 20 and 30 cycles. The amount of power required and the difficulty of obtaining ejaculation increase rapidly above 40 cycles.

It was also found that full erection of the penis could be easily obtained in the bull by the use of a sine wave pulse. However, erection is confined to a much more limited frequency range, 15 to 35 cycles for full erection. However, partial erection has been observed in some bulls at frequencies as high as 60 cycles. As with ejaculation, the best results were obtained between 20 and 30 cycles.

Method of stimulation. It is not generally necessary to hold the bull in a crush or chute. For most animals, all that is required is a stanchion similar to that used to hold the teaser cow for collection with the artificial vagina. To date, no trouble has been experienced in bringing the bull to the stanchion. The animals soon become conditioned for electric ejaculation and often have a partial erection as soon as they are brought near the stanchion. On cattle ranches a number of beef bulls have been electrically ejaculated for the first time with no more restraint than the use of a halter and a rope, which was tied to the nearest fence.

The technique as finally developed is as follows: The lucite electrode is placed in a bucket of warm water (which acts as a lubricant) and then fully inserted into the rectum of the bull. Stimulations are made by gradually increasing the intensity of the signal into the electrode over a period of 3 to 8 seconds and then returning to zero. Resting periods of 5 to 15 seconds are allowed between stimulations. As the treatment progresses, the intensity of the stimulation is increased. Erection and ejaculation usually occur in 1 to 3 minutes of intermittent stimulation. During the stimulating process, there is

a gradual peak voltage rise from zero to a maximum of 5.5 v. This is accompanied by a gradual increase in current up to 900 ma. at maximum excitation in the bull. It should be noted here that these recorded values are peak readings taken at maximum excitation and that under favorable conditions erection and ejaculation can often be obtained at a fraction of maximum output. This is especially true in bulls that have become accustomed to electric ejaculation and are conditioned to give the desired response.

With the electrode in position in the bull, the d.c. resistance varies from 300 to 450 ohms, depending on the amount of feces present. When, however, electric stimulations are commenced, the calculated impedance varies from approximately 16 ohms to 7 ohms as the current is increased. Since the phase angle has been found to be zero throughout the frequency range investigated, the reactance also is zero, and thus for practical purposes the load on the

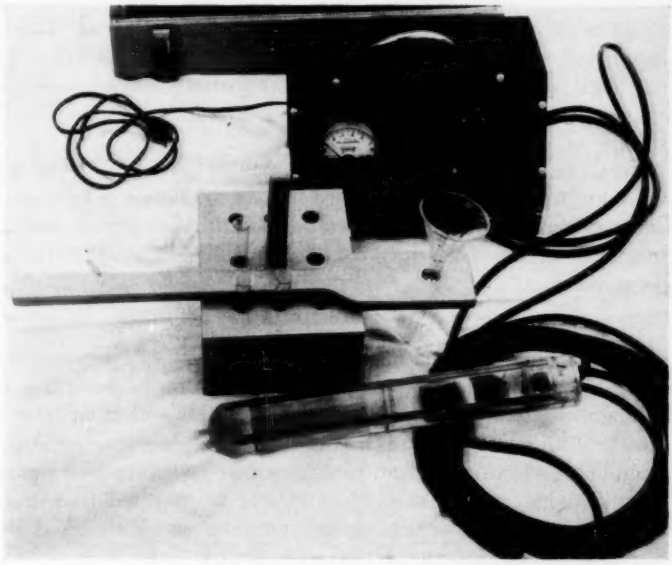

FIG. 1. Equipment used for electroejaculation.

electrode can be considered as purely resistive. As the power factor is equal to unity, it is apparent that 5 w. of power are required to be delivered by the generating source at maximum excitation. Ejaculation has frequently been obtained, however, at less than 2 w. power output in bulls accustomed to the technique.

Semen quality. As previously stated, experiments were carried out largely on two young bulls which were routinely electrically ejaculated from two to three times a week for a 12-month period. Ejaculates of good quality were routinely obtained. The volume of the ejaculate varied widely, depending on the quantity of accessory gland fluid collected and on whether single or multiple

ejaculates were obtained. Motility was generally excellent, as judged under the microscope. Unfortunately, no cows were available for insemination, and it was thus impossible to obtain fertility rates. However, Dziuk and Graham *(1)*, using equipment similar to that used by Laplaud *et al.*, obtained a 72.6% 30- to 60-day nonreturn rate from 530 cows inseminated from semen collected by electric ejaculation.

It should be mentioned here that electric ejaculation offers a method for the fractionation of the ejaculate. The secretions of the accessory glands are obtained during the initial part of the stimulating process. The true sperm-containing portion of the ejaculate is then obtained in a concentrated form following further excitation.

Ejaculation of bulls unable or unwilling to serve. In only two cases were we able to employ the apparatus on bulls which could be considered inhibited in their ability to serve. The first was a 17-year-old Brahma bull which had refused to serve naturally during the past two breeding seasons. Electrostimulation, though not causing erection, readily stimulated ejaculation. The semen was not of high quality but contained a surprisingly high concentration of motile cells in view of the age of the bull and the length of time since his last natural service.

The second case involved a Holstein bull reported by Hale *et al. (4)* as "generally inhibited." This bull showed no sexual desire toward any teaser animal. Electrostimulation of several minutes duration caused both erection and ejaculation to occur. The semen obtained was of poor quality but contained motile spermatozoa.

DISCUSSION

The method of electroejaculation described not only offers a simple method for obtaining ejaculates from bulls unwilling or unable to serve but also provides a technique which should prove useful for routine collection from vigorous young animals. The method has several obvious advantages over the use of the artificial vagina: *(a)* Routine collections can be made with the bull remaining on all four feet. This not only enables ejaculates to be collected from bulls which are unwilling to serve but also reduces the chance of accidents which sometimes occur when heavy bulls mount for semen collection. *(b)* The technique also eliminates the time lost with so-called slow bulls and provides a method that the stockman can use. *(c)* The instrument should prove of value to veterinarians who are required to examine ejaculates during sterility investigations on bulls unaccustomed to the use of the artificial vagina.

SUMMARY

A practical method of electric ejaculation has been developed which can be used routinely for the collection of semen for artificial insemination. Ejaculation is obtained without requiring the bull to mount a teaser animal.

A cylindrical electrode containing four longitudinal metallic strips is placed entirely inside the rectum. The electrodes are connected in pairs 180 degrees

apart. Ejaculation is obtained by use of an electric pulse. The wave form and frequency of this pulse are critical. Spike or square wave pulses will not cause either erection or ejaculation. The best results have been obtained by the use of a sine wave pulse. Ejaculation was obtained over a frequency range of 12 to 70 cycles, with the best results occurring between 20 and 30 cycles. Erection of the penis occurred in most bulls but its induction was limited to a frequency range of 15 to 35 cycles, with the best results having been obtained between 20 and 30 cycles.

Electric ejaculation causes a natural fractionation of the ejaculate. The accessory gland fluids are obtained first and can be discarded. The spermatozoa then can be obtained in a concentrated form.

No harmful effects have been observed in two bulls ejaculated routinely for a period of 12 months by this method.

REFERENCES

(1) Dziuk, P. J., and Graham, E. F. Application of Electroejaculation Technique to the Bull. *J. Dairy Sci.*, **36**: 598. 1953. (Abs. from ann. meeting.)

(2) Fulton, F. *Howell's Textbook of Physiology.* p. 1229. W. B. Sanders, Philadelphia. 1946.

(3) Gunn, R. M. C. Fertility in Sheep: Artificial Production of Seminal Ejaculation and the Characters of the Spermatozoa Contained Therein. Coun., Sci. Ind. Research, Australia, *Bull.* 94. 1936.

(4) Hale, E. B., Almquist, J. O., and Thacker, D. L. Observations on the Sexual Behavior and Semen Production of Dairy Bulls. *J. Dairy Sci.*, **36**: 576. 1953. (Abs. from ann. meeting.)

(5) Laplaud, M., Ortavant, R., and Thibault, C. La pratique de l'electroejaculation chez le Taureau. *Proc. First Intern. Congr. Physiol. and Pathol. of Animal Reproduction and Artific. Insem.* Malino, June, 1948.

(6) Thibault, C., Laplaud, M., and Ortavant, R. L. Electroejaculation chez le Taureau. *Extrait des comptes rendus des seances de l'Academie des Sciences*, t 226. p. 2006. June, 1948.

OUTDOOR INDIVIDUAL PORTABLE PENS COMPARED WITH CONVENTIONAL HOUSING FOR RAISING DAIRY CALVES

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New-born calves are often housed in areas contaminated by other calves or older cattle. Under these conditions the mortality rate frequently is very high. Often, high losses occur in herds where even better than average management and housing facilities are available *(1, 12)*. It has been reported that the use of outdoor, individual portable pens that are moved once each week to break the life cycle of coccidia is an aid in keeping young calves relatively free from clinical coccidiosis, as well as other diseases *(4)*. A comparison of these pens with four other types of enclosures over a 9-year period under laboratory conditions revealed that calves in the portable pens had lower morbidity and mortality rates and required less labor than calves in the other enclosures *(6)*.

An open shed system of raising healthier dairy calves has been recommended for winter use in Maryland *(3)*, Pennsylvania *(11)*, Wisconsin *(13)*, and New York *(9)*. Erb *et al. (7)* have reported reduction in morbidity and mortality by using open sheds in Washington, even when temperatures went as low as −20° F.

No controlled experiments, except the ones conducted with calves artificially infected with coccidia at the Regional Laboratory, had been made to compare the portable pens with a conventional system under farm conditions. Consequently, in the fall of 1950, the study reported here was initiated at the dairy farm of the Alabama Polytechnic Institute in order to compare growth responses and parasitism of calves in the two systems of management.

EXPERIMENTAL

Calves born in September and October, primarily of Jersey breeding but including a few Holstein × Jersey and Guernsey × Jersey crosses, were the experimental subjects. During each of two successive years, 16 calves less than 3 days of age were randomly assigned to two housing and management groups of eight animals each. The systems of housing and management were: *(a)* conventional and *(b)* outside individual portable pens. The calves remained in the two types of housing until they were an average age of 149 days during the first year, and until each was 6 months old during the second year. They were then placed together on pastures. Calves assigned to the conventional

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FIG. 1. Calf barn and exercise lot used in this experiment.

system were housed in individual stalls with concrete floors and solid partitions, inside a barn. The stalls were cleaned twice each week and bedded with shavings. When weather permitted, these calves were turned into a 15 by 40 ft. lot sparsely covered with vegetation (Figure 1). Calves assigned to the outdoor individual pen group were each placed in a 5 by 10 ft. enclosure (Figure 2). One half of each portable pen was covered at all times and protected on three sides during the winter; a small amount of straw bedding was used in the severest weather. Once each week the pens were moved in the recommended manner *(5)*. During

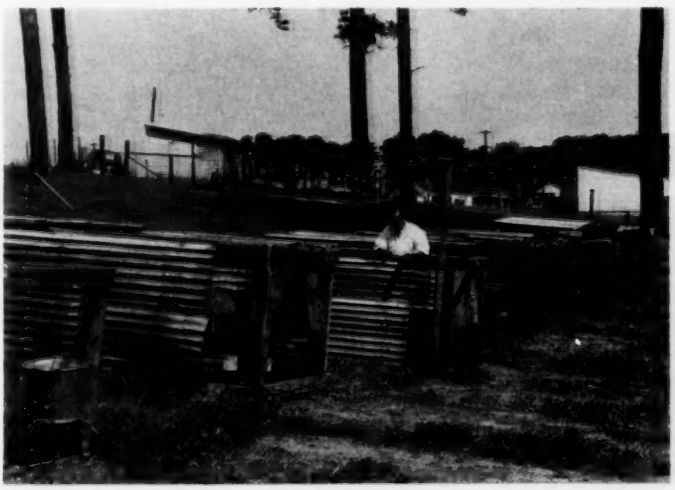

FIG. 2. Part of a row of eight portable pens partly enclosed for winter use. White building in right background is the calf barn used in this experiment.

the first year, the portable pens were placed in a 1-acre lot that was comparatively free of contamination by older animals, having been plowed and isolated for 2 months. During the second year, the lot was contaminated by older cattle that entered the area accidentally before the calves were placed in the portable pens. Forty-four dung pads were removed, but rains had scattered some of the contaminating material.

All calves received the same diets except for the limited grass available to calves in the portable pens and the small amount of vegetation in the exercise lot. Whole milk was fed at the rate of 1 lb. daily per 12 lb. of body weight until the calves were 6 weeks old, at which time whole milk in the ration was replaced by a milk substitute containing 10% nonfat dry milk solids. When the calves were 4 months old, the milk substitute was replaced by a concentrate mixture containing 16% protein. From 10 days of age, all calves received as much alfalfa hay (approximately U.S. No. 2) as they would eat. All calves had access to water that was replenished at least twice daily.

During the second year, in order to obtain information on the possible importance of rumen organisms on the response of calves in the first year of this investigation, rumen fluid from cows on pasture was given to four calves in each housing and management group when they were 4 weeks old.

On two successive days, at birth and once each 4 weeks thereafter, the calves were weighed and heart girth, barrel girth, and height at withers were measured, except for the first year when barrel girth measurements were started at 30 days of age. Because of the death of one barn calf, only the growth data collected prior to 120 days of age were subjected to statistical treatment.

Starting when the calves were 2 weeks of age, feces from each calf were collected three times every week and examined by Boughton's technique *(2)* for estimating number of oocysts of coccidia. The incidence of diarrhea was determined from the same samples. Once each week a fresh fecal sample from each calf was examined for numbers of eggs and species of worm parasites by using the direct centrifugal flotation technique and recording the results in eggs per gram (EPG) of fecal material. Once each month after the calves were moved onto pastures, they were examined for oocysts of coccidia and worm eggs.

A stomach pump was used to obtain rumen contents from each calf before it was placed on pasture. The presence of rumen ciliates was used only as an indication that the calf had been exposed to rumen flora and fauna from other cattle; however, no rumen bacteria were cultured. During the first year the calves were moved to pasture when the average ages of the barn calves and portable pen calves were 148 and 150 days, respectively. In the second year, all calves were moved to pasture at 6 months of age.

RESULTS

Examination of rumen contents at the time the calves in the first year's test were moved to pasture showed that none of the calves in the portable pens had acquired rumen protozoa, whereas all of the barn calves had these ciliates. When

the calves in the second year were placed on pasture, examinations showed that all calves in the barn had rumen protozoa, and all of those in the portable pens had rumen ciliates with the exception of two of the four calves that had not been inoculated with rumen contents.

Although the baby calves in the portable pens were subjected to outside temperatures as low as 9° F. in the fall of 1950 *(10)*, no adverse effects were observed. There were no signs of frostbitten ears. In both years every calf in the barn showed respiratory troubles, chronic spasmodic coughing, nasal discharges, abnormal respiration, general weakness, and other symptoms of early pneumonia. During the first year one calf in the barn died from pneumonia at 16 weeks of age following an earlier attack by *Eimeria zurnii*, which is one of the most pathogenic coccidia affecting cattle. Only one calf in the portable pens showed signs of respiratory difficulties, and these were limited to a slight cough and weakness that persisted for only 1 week.

Diarrhea. During both years, the barn calves were diarrheic more times than the calves in the portable pens. During the second year, in the barn, the uninoculated calves were diarrheic more than twice as many times as the rumen-inoculated ones. In the portable pens, the rumen-inoculated calves were diarrheic slightly more times than the uninoculated ones.

Coccidia. In the first year, during the period between the second and 18th weeks of age, the portable pen calves were negative for oocysts of coccidia a total of 171 times as compared with only 50 times in the barn calves. The portable pen calves showed counts between 1,000 and 4,999 oocysts per loopful on 14 examinations, and the barn group fell within this range 28 times. Counts of over 1,000 oocysts per loopful were sometimes accompanied by symptoms of coccidiosis, agreeing with a previous report *(2)*. Only one calf in the portable

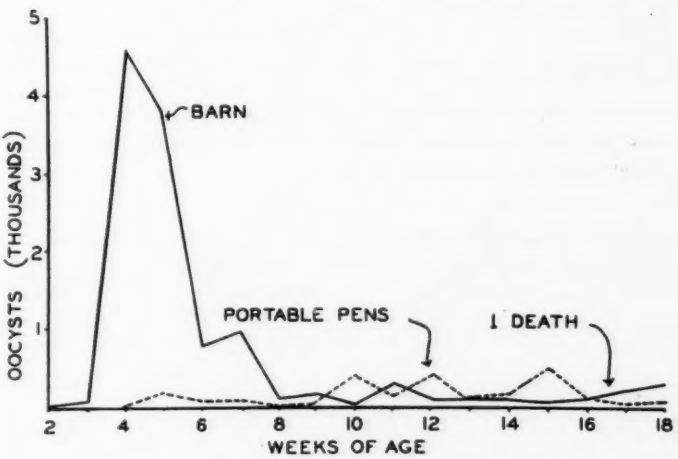

FIG. 3. Comparison of output of oocysts of coccidia from calves raised in a barn and calves raised in portable pens during the first year when the portable pens were on un-contaminated ground.

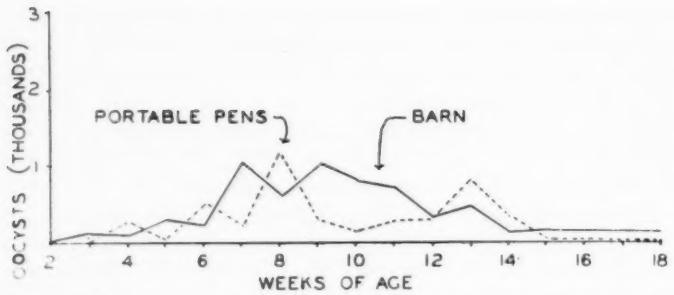

FIG. 4. Comparison of output of oocysts of coccidia from calves raised in a barn and calves raised in portable pens during the second year when the portable pens were on contaminated ground.

pen group had an oocyst count of over 5,000, whereas five of the barn calves had this number from 1 to 3 days each, for a total of 11 times. None of the portable pen calves had counts of over 10,000 oocysts per loopful, but the barn-group had this number six times. Between the third and seventh weeks of age, the barn calves discharged about 30 times as many oocysts as those in the portable pens (Figure 3). Results of the second year's test are indicated in Figure 4 and Table 1.

Oocysts of ten species of coccidia were found in each of the two groups of calves. In both groups, *Eimeria zurnii* appeared the greatest number of times, with *E. ellipsoidalis*, *E. auburnensis*, and *E. bovis* occurring in progressively decreasing numbers, respectively. *E. zurnii* and *E. bovis*, which are the most destructive species affecting cattle in this area *(5)*, were present as described below.

In the first year, counts of over 1,000 oocysts of *E. zurnii* occurred in two calves in the portable pens on one examination each. Similar counts were found in six calves in the barn seven, eight, one, three, one, and one times, respectively. During the same year, counts of over 1,000 oocysts of *E. bovis* were found in two barn calves, one and five times, respectively. No portable pen calves showed counts of over 1,000 oocysts of this species.

TABLE 1

Oocyst counts from second to 18th weeks in rumen-inoculated and uninoculated calves assigned to two systems of management

Oocyst counts	Uninoculated		Inoculated		Totals	
	Conv. housing	Portable pens	Conv. housing	Portable pens	Uninoculated	Inoculated
No. times no oocysts	18	26	43	52	44	95
No. times 1,000-5,000 oocysts [a]	26	21	12	6	47	18
No. times over 5,000 oocysts [a]	0	2	1	0	2	1
Mean daily oocyst counts [a]	465	395	348	172	860	520

[a] Oocysts per loopful, as determined by Boughton's technique *(2)*.

During the second year, the incidence of *E. zurnii* and *E. bovis* was similar to that of the first year, a higher rate of infection with both species being found among the barn-raised calves.

In both years there was no evidence of difference in susceptibility to coccidia of the two groups of calves after they were placed together on pasture. In neither group on pasture was clinical coccidiosis observed; oocysts counts remained low.

Parasitic worms. On the basis of fecal egg counts, during the first year, the barn calves became infected with nematode parasites much sooner and in greater numbers than did the portable pen calves. All of the barn group became infected at an average age of 9.5 weeks, whereas only half of the portable pen group became infected at an average age of 21 weeks. The others in the pens did not acquire worms until the calves were placed on pasture. The nematodes involved were *Haemonchus contortus, Trichostrongylus axei, Ostertagia ostertagi,* and *Cooperia punctata.* Egg counts exceeding 60 per gram of feces (EPG) were not observed in either group, indicating that none of the animals had acquired enough worms to impair health.

Three weeks after the animals were placed on pasture, during the first year, all were passing eggs of the nematode species listed above. After 4 months of grazing, the average fecal egg count of each group was about 200 EPG. The level of parasitism was low, and there was no difference in susceptibility to worm parasites between the pastured calves raised in portable pens and those raised in the barn.

In the second year, all calves in both systems of management became lightly infected with one or more species of parasitic worms. The average fecal egg count per gram for each group did not exceed 50, and neither group showed enough worm parasitism to require treatment. When the calves were placed on pasture, the fecal egg count in all calves began to rise, but in no case did it exceed a peak of 250 per gram.

Growth. The mean gains in body weight, in height at the withers, in heart

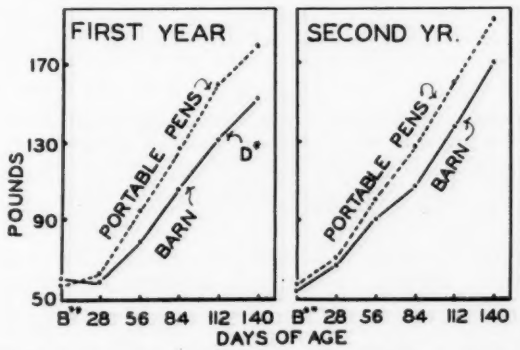

FIG. 5. Comparison of average weight gains of calves raised in portable pens and calves raised in a calf barn.

* Death of one calf in the barn group.

** Average weights at time of birth.

TABLE 2

Mean gains in body weight, height at withers, heart girth, and barrel girth of young dairy calves, during 120-day periods,[a] associated with two systems of management

Measurement	Mean gains, by years, associated with management		
	Conv. housing	Portable pens	L.S.D.[b]
Body weight *(lb.)*			
1950-51	70.0	101.3	20.9[c]
1951-52	85.8	103.6	17.2[d]
2-yr. av.	77.9	102.4	14.8[c]
Height at withers *(in.)*			
1950-51	4.7	5.3	1.0[d]
1951-52	4.9	5.6	0.6[d]
2-yr. av.	4.8	5.5	0.5[d]
Heart girth *(in.)*			
1950-51	9.1	11.8	2.4[c]
1951-52	9.3	10.7	1.6[d]
2-yr. av.	9.2	11.3	1.5[c]
Barrel girth *(in.)*			
1950-51	13.4	17.3	3.5[c]
1951-52	12.8	15.5	3.4[d]
2-yr. av.	13.1	16.4	2.9[c]

[a] Barrel girth data are for the period 30 to 120 days of age.
[b] Least significant difference between means.
[c] $t = 0.01$.
[d] $t = 0.05$.

girth, and in barrel girth of the calves from birth to 120 days of age are illustrated in Table 2. Gains in body weight of portable pen calves during each year of the study were significantly greater ($P < 0.01$) than those of the calves raised in the barn. Also, the combined data for the 2 years of the study indicate that calves assigned to the portable pens made significantly greater gains in body weight ($P < 0.01$), in height at the withers ($P < 0.05$), in heart girth ($P < 0.01$), and in barrel girth ($P < 0.01$). The growth curves for both groups of calves during the two-year period are shown in Figure 5.

The growth response of calves that were fed rumen fluid did not differ significantly from that of calves that did not receive rumen fluid (Table 3).

TABLE 3

Relationship between rumen inoculation of young dairy calves assigned to two systems of management and mean gains in body weight, height at withers, heart girth, and barrel girth from birth to 120 days of age

Measurement	Mean gains associated with treatments				
	Conv. housing		Portable pens		
	Uninoculated	Inoculated	Uninoculated	Inoculated	L.S.D. $t = 0.05$
Body weight *(lb.)*	83.5	88.0	106.8	100.5	26.5
Ht. at withers *(in.)*	4.7	5.1	5.7	5.5	0.9
Heart girth *(in.)*	9.0	9.7	11.0	10.5	2.4
Barrel girth *(in.)*	16.8	18.7	20.4	17.3	3.0[a]

[a] Application of the "F" test revealed that the differences in gains in the barrel girths were not significant.

When the calves were moved to pasture during the first year at an average age of 5 months, the ones from the portable pens weighed an average of 181 lb., whereas the average weight of the barn calves was 150 lb. After remaining together for 2 months, the ones that had been raised in the portable pens weighed an average of 275 lb., as compared with 237 lb. for the barn-raised calves. This phase of the experiment was terminated at that time.

In the second year, when the calves were placed together on pasture at the age of 6 months, the portable pen calves weighed an average of 229 lb., as compared with an average of 200 lb. for the barn calves. Two months after being on pasture, the calves from the pens weighed an average of 297.6 lb., and the barn-raised ones weighed an average of 261 lb. Ten months after being placed on pasture, the animals from the portable pens weighed an average of 510 lb., as compared with an average weight of 482 lb. for the other group.

DISCUSSION

The results of this investigation indicate that under similar ecological conditions healthier calves may be raised and a lower mortality may be expected when the portable pen system is used in place of the more conventional plan of housing calves in a barn. There is a possibility that the ad libitum feeding of grain and hay did not facilitate the establishment of balanced rumen flora and fauna; however, both groups of calves were fed by the same procedure. It is possible that the additional weight gain (24.5 lb.) of the portable pen calves at 120 days of age as compared with the barn calves (Table 2) could have been due to differences in quantity of grass available. However, both groups received an adequate supply of high quality hay, and it was observed that when a portable pen was moved to a fresh site, there was practically no edible grass available after the first day. The exercise lot furnished a small amount of vegetation for the barn calves. Results of a third year's work (unpublished) indicate that the limited amount of winter grass was an unimportant factor in the superior gains made by calves in the portable pens.

In considering the growth rate of calves under the two systems of management, it may be of interest to note that the purebred Jersey calves in the outside pens of this study generally were very alert and vigorous in appearance, when compared to barn-raised calves. However, the outside calves were 4% shorter in height at withers and 9% lighter in body weight at 4 months than Jersey calves by Ragsdale standards *(8)*. This difference may be due partly to the strain of Jerseys in the Alabama Station herd. All calves in this study were raised on a limited milk regime, which fact undoubtedly contributes to this slight difference between outside calves and Ragsdale standard calves in size at 4 months.

Despite the similarity of counts of oocysts of all species of coccidia in both groups of calves during the second year, the greater incidence of infections with the most destructive species, *E. zurnii* and *E. bovis*, in the barn calves each year could be one of the reasons for smaller gains in that group as compared with the portable pen calves. The presence of chronic respiratory troubles in

every calf in the barn and the almost complete absence of these troubles (except in one calf) in the ones in the portable pens was probably the greatest factor involved in the weight gains.

SUMMARY

Thirty-two new-born calves were divided equally and placed in individual outdoor portable pens or in a barn and exercise lot during a test covering 2 years. During the second year, half of the calves in each system of management were given inoculations with rumen fluid. The pen calves were placed on a site which was clean the first year and slightly contaminated by older animals the second year. Despite temperatures as low as 9° F., the calves in the portable pens made significantly greater weight gains both years and had fewer coccidia and worm parasites and less diarrhea than the barn calves. All calves in the barn had respiratory troubles, but only one calf in the pens had this trouble. One barn calf died from pneumonia following an attack by *E. zurnii*, a pathogenic coccidian. The growth response of calves that were fed rumen fluid did not differ significantly from that of uninoculated calves.

When placed together on pasture at the age of 5 or 6 months, the portable pen calves showed no more susceptibility to coccidia and worm parasites than the calves from the barn and continued to maintain their superior weights.

REFERENCES

(1) Autrey, K. M. Unpublished data collected in 1942 at University of Georgia.

(2) Boughton, D. C. Sulfaguanidine Therapy in Experimental Bovine Coccidiosis. *Am. J. Vet. Research*, 4: 66. 1943.

(3) Buckley, S. S. Open Stables Versus Closed Stables for Dairy Animals. Md. Agr. Expt. Sta., *Bull.* 177. 1913.

(4) Davis, L. R. Portable Pens for Raising Healthy Dairy Calves. Mimeo., U.S.D.A. Jan. 1949; rev., Sept. 1949.

(5) Davis, L. R., and Bowman, G. W. Coccidiosis in Cattle. *Proc. 55th Ann. Meeting, U. S. Livestock San. Assoc.*, Kansas City, Mo. 1951.

(6) Davis, L. R., Bowman, G. W., and Porter, D. A. Portable Pens Compared with Other Enclosures for Control of Diseases of Dairy Calves. *Vet. Med.*, 47: 485. 1952.

(7) Erb, R. E., Gilden, R. O., Goodwin, M. M., Millard, J. B., and Murdock, F. R. Open Shed Versus Conventional Housing for Dairy Calves. Wash. Agr. Expt. Sta., *Tech. Bull.* 3. 1951.

(8) Ragsdale, A. C. Growth Standards for Dairy Cattle. Mo. Agr. Expt. Sta., *Bull.* 336. 1934.

(9) Udall, D. H. "Sulfathalidine" Phthalylsulfathiozole in the Prevention and Treatment of Diarrhea in Calves. *North Am. Veterinarian*, 30: 581. 1949.

(10) U.S. Weather Bureau. Alabama climatological data. 1950.

(11) Watts, R. L. The Effect of Open-Shed Housing as Compared with the Closed Stable for Milking Cows. Pa. Agr. Expt. Sta., *Bull.* 147. Director's Report, 1916-1917.

(12) Weaver, E., Horwood, R. E., and Smiley, E. S. Losses of Calves in Dairy Herds. *Mich. Quart. Bull.* 32. p. 42. 1949.

(13) Witzel, S. A., and Heizer, E. E. A Five Year Summary of Dairy Barn Research. *Agr. Eng.*, 27: 499. 1946.

THE EFFECT OF CATION AND ANION RESIN MIXTURES ON THE SALT CONTENT OF RAW SKIMMILK

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During the processing of milk and its products, milk proteins are exposed to a wide variety of treatments, such as homogenization, heating, freezing, and storage, which tend to destabilize them. The ionic composition of the products is one of the important factors that influence the stability of the milk proteins and therefore determine the quality of the finished product. In many cases to obtain a suitable product after treatment, the ionic composition of the milk must be controlled or modified. This may be accomplished by treatment with mixtures of cationic and anionic exchange resins. In order to increase our knowledge concerning this method, the effects of mixtures of Nalcite HCR (a sulfonated copolymer of styrene and divinyl benzene) and Nalcite SAR (a quaternary amine resin) on the salt content of raw skimmilk have been studied.

The modern concept of ion exchange was derived from the work of Way *(30)* and Thompson *(27)* on clays. A comprehensive review of the subject has been presented by Boyd *(6)*. The principle of ion exchange was first applied for the modification of the ionic composition of milk by Lyman, Browne, and Otting *(19)*, using an inorganic base exchange process. This treatment has been found useful in the preparation of low ash casein, in the retardation of sandiness, and the improvement of the whipping qualities of the ice cream.

In recent years, the discovery of synthetic ion exchange resins by Adams and Holmes *(1)* made possible their use in many fields. They have been widely used for the modification of calcium and phosphorus in milk *(8, 23)*. The removal of calcium has been found to be influenced by the pH of the milk samples *(31)*. Fundamental studies on the effect of ion exchange resins on simple salt systems simulating those in milk and solutions of lactose and nonprotein nitrogen have been made by Gehrke and Almy *(12)*. Haller and Morin *(14)* have suggested that the extent and kind of ion exchange modification in milk could be controlled by the selection of proper combinations of exchangers and by the method used for the operating cycle.

Addition of ion exchange treated milk has been found to stabilize evaporated milk during sterilization *(16)*, retard the flocculation of proteins of concentrated milk in the frozen state *(30)*, and improve the stability of the frozen milk *(13)*. The process of ion exchange has been used for the maximum recovery of lactose from whey *(2, 20)*, to produce dehydrated cream which could be used in coffee

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[1] The data published here are taken from a thesis presented by the senior author to the faculty of the Graduate School, University of Illinois, in partial fulfillment of the requirements for the Master of Science degree, June, 1953.

without feathering *(10)*, to determine the calcium ion activity in milk *(9)*, and for the hydrolysis of casein *(28)*. Resin-treated whey has been used in the preparation of food products *(21, 22)*. However, not much information is available as to the effect of cation and anion resin mixtures on the salts of milk.

EXPERIMENTAL

Resin regeneration procedure. Since the cation resin, Nalcite HCR, was in the sodium form and the anion resin, Nalcite SAR, was in the chloride form, it was necessary to regenerate them on the hydrogen and the hydroxyl cycles, respectively. The regeneration of the cation resin was performed by shaking about 25 g. of the resin with 100 ml. of 5% hydrochloric acid on a mechanical shaker (New Brunswick Scientific Co., model VS 5035) at 240 r.p.m. for 10 minutes, and the anion resin was shaken with 5% sodium hydroxide in a similar manner. This procedure was repeated thirteen times for cation resin and fifteen times for anion resin for the complete displacement of sodium and chloride, respectively, as indicated by preliminary experiments.

The resulting cation and anion resins then were washed nine times with successive 100-ml. portions of deionized water by shaking on the mechanical shaker at 240 r.p.m. for 10 minutes to remove all the excess of acid or alkali, respectively, as indicated by the constancy of the pH values of the washings.

Determination of the replaceable hydrogen ion and hydroxyl ion content of the cation and the anion resins. In order to specify the amount of cation or anion resin used in the treatment of the milk, it seemed desirable to express it in terms of milliequivalents of replaceable hydrogen ions or hydroxyl ions on the resin, respectively, rather than in terms of the weight of the resin, because of its uncertain moisture content. The cation or anion resin regenerated as described previously was spread on paper (6.5 in. × 10 in.) and dried with the aid of a fan for 45 minutes with occasional mixing. Then 5 g. of the cation (or anion) resin was accurately weighed and titrated against 0.5 *N* sodium hydroxide (or 0.5 *N* hydrochloric acid) with 50 ml. of 1 *N* sodium chloride as a supporting electrolyte. From the titration values the number of milliequivalents of hydrogen ions (or hydroxyl ions) per gram of resin was calculated. In general, a value of 2.70 meq. of hydrogen ions (or 1.50 meq. of hydroxyl ions) per gram of air-dried resin was obtained.

Treatment of raw skimmilk. Preliminary studies of the effect of sample size and time of shaking on the achievement of equilibrium as indicated by pH values showed that in order to avoid variations due to sample size and to reach equilibrium the samples should be shaken for 60 minutes.

Several samples of fresh raw skimmilk were obtained from the University Creamery, adjusted to 25 ± 1° C., and treated with cationic and anionic resin mixtures at four different levels each. Resin mixtures containing 0.00, 7.47, 14.94, and 22.41 meq. of hydrogen ions and 0.00, 18.66, 37.32, and 55.98 meq. of hydroxyl ions in all possible combinations were shaken with 400-ml. portions of milk on the mechanical shaker at 240 r.p.m. for 60 minutes. The samples were

filtered through cotton wool and analyzed for pH, total nitrogen, calcium, magnesium, sodium, potassium, total phosphorus, inorganic phosphorus, citric acid, sulfate, and chloride.

Analytical methods. Throughout this investigation the following analytical methods were employed:

1. The pH was determined by using a Beckman model G pH meter with glass electrode (No. 290) and calomel electrode (No. 270). Sodium corrections were applied to the pH values when necessary.

2. The total nitrogen content was measured by direct nesslerization of the digested sample *(15)*.

3. Calcium and magnesium were determined in the ashed sample *(26)* by the potassium palmitate method *(3)*.

4. Total phosphorus was estimated by means of Verma's technique *(29)* for digesting the milk samples and Fiske and Subbarow's method *(11)* for color development.

5. Inorganic phosphorus was measured in an aliquot of the filtrate obtained from trichloracetic acid precipitation of the proteins in the milk sample *(26)* by developing the color with Fiske and Subbarow's method.

6. Citric acid was estimated gravimetrically by the method of Lampitt and Rooke *(18)*.

7. Sulfate was determined in the ashed sample gravimetrically *(5)*.

8. Sodium was measured in the ashed sample as sodium uranyl zinc acetate *(17)*.

9. Chloride was estimated as silver chloride according to A.O.A.C. methods *(4)* with the following modifications: 2 ml. of 6% (w/v) $KMnO_4$ was added before and a pinch (0.25 g.) of urea was added after digesting the sample, and 6 ml. of acetone was added to the solution before titration.

10. Potassium was determined in the ashed sample according to the method proposed by Bray *(7)* modified for volumetric measurement. The standard potassium solution was made up to correspond to the composition of the milk ash solution by dissolving 0.6268 g. of $Ca_3(PO_4)_2$; 0.0306 g. of MgO; 0.7236 g. of Na_2HPO_4; 0.0827 g. of Na_2CO_3; 0.6091 g. of KCl; and 19.68 ml. of HCl in water and making up the volume to one liter. Ten-ml. aliquots of the ashed sample, the standard solution, and the blank were heated separately with 1 ml. of sulfuric acid (1:1) on a hot plate until white fumes appeared, 0.5 ml. of concentrated nitric acid was added to each, and the resulting solutions were ignited to whiteness over a burner. After cooling, each of the residues was dissolved in 1 ml. of concentrated nitric acid and 20 ml. water. Twenty ml. of sodium acetate was added to each, and the mixtures were cooled to 15-20° C. Potassium was precipitated by adding a mixture prepared by mixing 15 ml. of cooled cobaltic nitrite reagent and 25 ml. of cooled 95% alcohol. The precipitates were filtered through sintered glass crucibles and washed, first with acidified sodium acetate and alcohol mixture (1:1), then with alcohol, and finally were dried with ether. They then were oxidized with 10 ml. of 0.1 *N* potassium permanganate and dissolved in 7 ml. of 1:1 sulfuric acid and 50 ml. of water by

warming the solutions to 70° C. Ten ml. of 0.1 *N* sodium oxalate was added and the solutions were back titrated with 0.1 *N* potassium permanganate solution.

RESULTS

The results obtained on a typical milk are illustrated in Figures 1 to 11. In addition, the total concentrations of the cations on the Nalcite HCR resin after treatment are indicated in Figure 12.

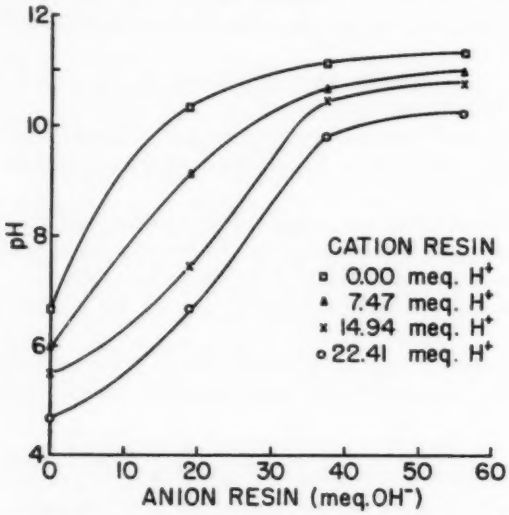

FIG. 1. Effect of resin mixtures of Cation HCR (H^+ cycle) and Anion SAR (OH^- cycle) on the pH of raw skimmilk.

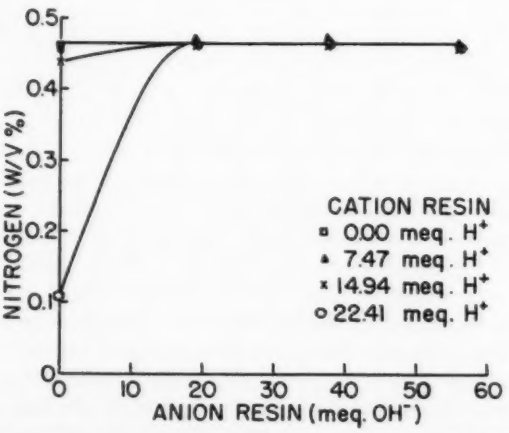

FIG. 2. Effect of resin mixtures of Cation HCR (H^+ cycle) and Anion SAR (OH^- cycle) on the nitrogen content of raw skimmilk.

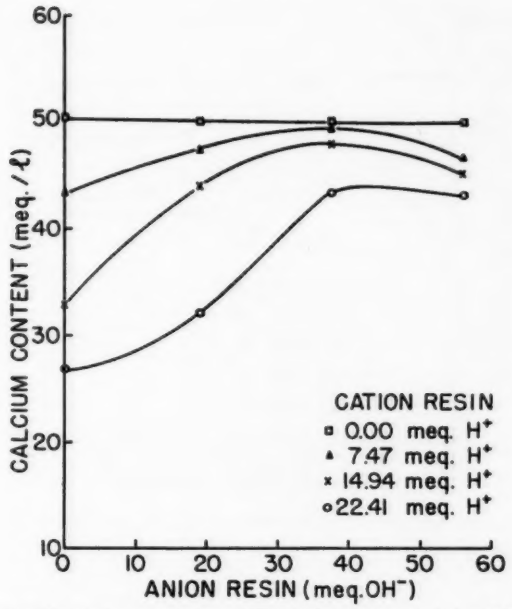

FIG. 3. Effect of resin mixtures of Cation HCR (H^+ cycle) and Anion SAR (OH^- cycle) on the calcium content of raw skimmilk.

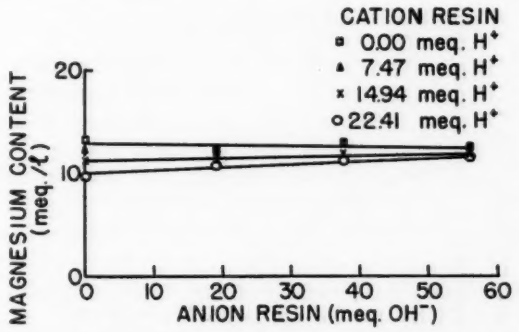

FIG. 4. Effect of resin mixtures of Cation HCR (H^+ cycle) and Anion SAR (OH^- cycle) on the magnesium content of raw skimmilk.

DISCUSSION

Statistical analysis of the results indicates that the effect of mixtures of exchange resins upon the salt content of the milk is significantly (0.1% level) influenced by the nature of the specific milk sample under consideration. However, these influences are more quantitative than qualitative. Therefore, the figures presented satisfactorily represent what can be expected under the different treatments except for unusual milk.

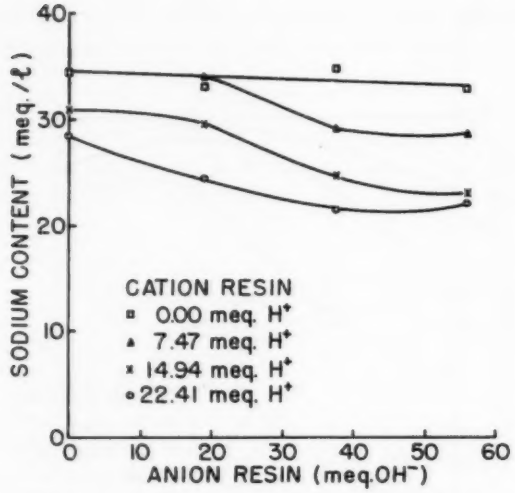

FIG. 5. Effect of resin mixtures of Cation HCR (H^+ cycle) and Anion SAR (OH^- cycle) on the sodium content of raw skimmilk.

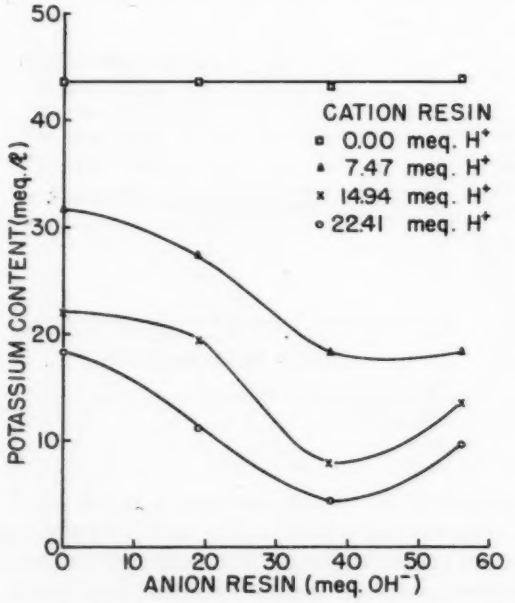

FIG. 6. Effect of resin mixtures of Cation HCR (H^+ cycle) and Anion SAR (OH^- cycle) on the potassium content of raw skimmilk.

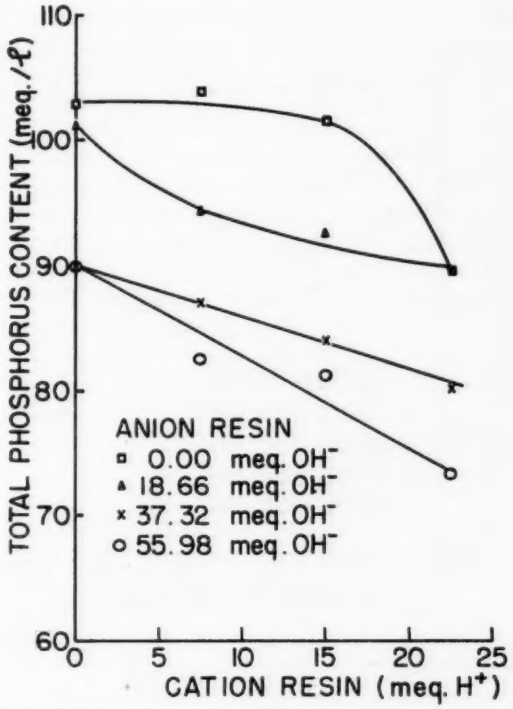

FIG. 7. Effect of resin mixtures of Anion SAR (OH^- cycle) and Cation HCR (H^+ cycle) on the total phosphorus content of raw skimmilk.

From Figure 2, it can be seen that no nitrogenous material is removed from the milk by this treatment except when the cation resin is used alone at the two higher levels. Under these conditions, the equilibrium pH is 5.5 or below and, therefore, some protein coagulation with subsequent removal on filtration may be expected.

Upon inspection of all of the figures it can be seen that there is no significant removal of cations from the milk when treated by anionic resin alone and no removal of anions when treated with cationic resin alone.

Consideration of Figures 3, 5, and 6 indicates the interrelationship between calcium, sodium, and potassium removal. At any one cationic resin level, as the amount of anionic resin is increased, the calcium removal decreases to a minimum and then increases, whereas the combined sodium and potassium removal increases until the maximum is reached at approximately the same amount of anionic resin and then decreases. As indicated by the work of Boyd *(6)*, these ions should be preferentially adsorbed in the following order: $Ca^{++} > K^+ > Na^+$ if they are present as free ions. Since the variation in the relative amounts of the resins present in the mixture or their effect on the milk would

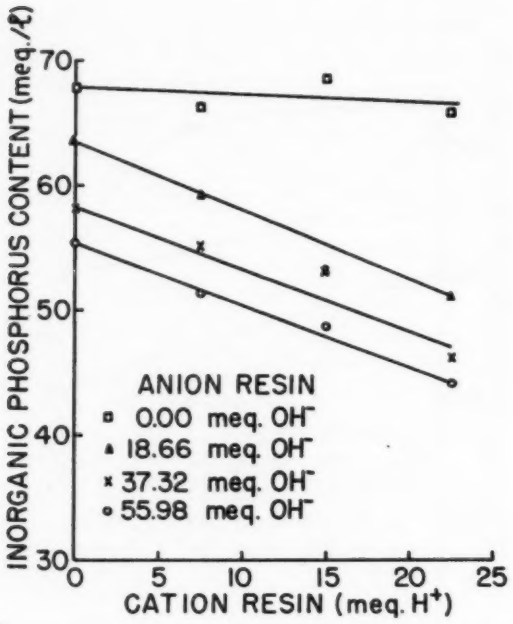

FIG. 8. Effect of resin mixtures of Anion SAR (OH^- cycle) and Cation HCR (H^+ cycle) on the inorganic phosphorus content of raw skimmilk.

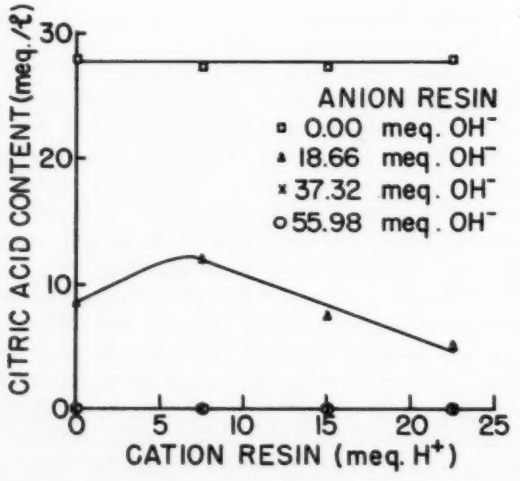

FIG. 9. Effect of resin mixtures of Anion SAR (OH^- cycle) and Cation HCR (H^+ cycle) on the citric acid content of raw skimmilk.

not be expected to influence the adsorptive character of the cationic resin, the observed effects seem to indicate that the calcium present is increasingly complexed in some manner as the amount of anionic resin is increased until the minimum is reached. This may be due to the changes produced in the pH of the milk sample. With the removal of the free calcium ions from the system in this fashion, more sodium and potassium can be adsorbed by the cation resin. More evidence is needed on this phenomenon.

The effect of the resin mixture upon the magnesium content of skimmilk, as illustrated by Figure 4, is interesting. Although statistical analysis of the results indicates that the milk sample, the anion level, and the cation level significantly (0.1% level) affect the magnesium content of the sample, the changes are small and varied. No ready explanation of the phenomenon is available. It may be the preferential adsorbability of magnesium is such on Nalcite HCR that the amount of it removed is chiefly dependent upon the amount of calcium, sodium, and potassium present.

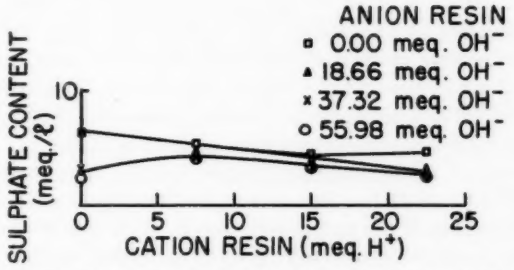

FIG. 10. Effect of resin mixtures of Anion SAR (OH^- cycle) and Cation HCR (H^+ cycle) on the sulfate content of raw skimmilk.

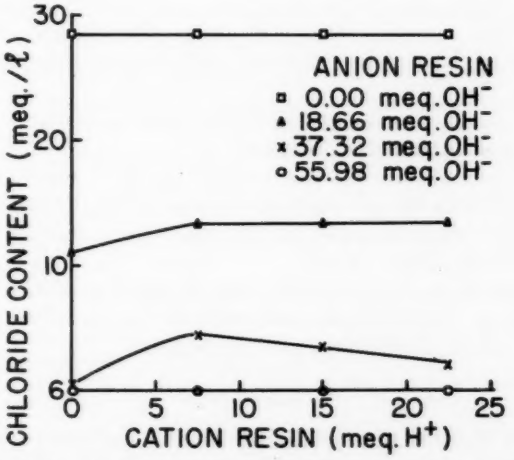

FIG. 11. Effect of resin mixtures of Anion SAR (OH^- cycle) and Cation HCR (H^+ cycle) on the chloride content of raw skimmilk.

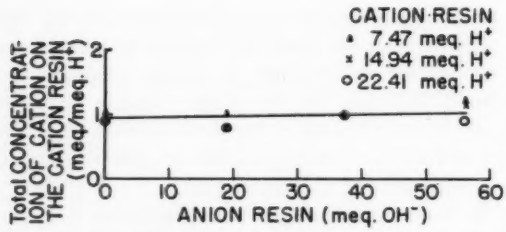

FIG. 12. Total concentration of cations on the Cation HCR resin after treatment of raw skimmilk with resin mixtures of Cation HCR (H^+ cycle) and Anion SAR (OH^- cycle).

Consideration of Figures 7 and 8 indicates that, except for the sharp reduction of total phosphorus when the highest level of cationic resin is employed alone and coagulation occurs, the amounts of total phosphorus and inorganic phosphorus removed are in qualitative agreement. The greater removal of phosphorus at higher levels of cationic resin is to be expected, since the ionic forms of phosphorus present at the lower pH values obtained at these levels would neutralize fewer positive charges on the anion resin per equivalent of phosphate removed, than those present at the higher pH values. The lack of correlation between the calcium and phosphorus removed indicates that the possibility of removal of calcium as a calcium phosphate complex is slight.

The adsorption of citrate ions by Nalcite SAR is very strong, as indicated in Figure 9. All the available citrate ions are removed at the second level of anionic resin regardless of the cation resin level. With smaller amounts of anion resin, the cation resin level appears to have an effect similar to its effect on phosphate ion removal.

The effect of the resin mixture upon the sulfate content of raw skimmilk, as indicated in Figure 10, is difficult to interpret, since the differences between treatments, although statistically significant, are small and irregular. It may be that an explanation similar to that proposed for magnesium applies to sulfate ions as well.

The adsorption of chloride ions by Nalcite SAR is similar to that of citrate although weaker, as indicated in Figure 11.

Figure 12 shows that, at the levels of Nalcite HCR resin employed in this study, the cations from the raw skimmilk have completely replaced the hydrogen ions in the resin. Statistical analysis of these values indicates no significant deviations from unity in any of them.

A more quantitative explanation of these results is desirable, but basic information concerning the equilibrium constants of the resins is necessary before this is possible. Work in this direction is in progress.

SUMMARY

The action of mixtures of cationic (Nalcite HCR, hydrogen cycle) and anionic (Nalcite SAR, hydroxyl cycle) resins upon the salt content of skimmilk has been investigated. Raw skimmilk samples were treated by the batch process with

the resin mixtures, and the resulting systems were filtered and analyzed for pH, total nitrogen, calcium, magnesium, total phosphorus, inorganic phosphorus, citrate, sodium, potassium, and chloride. Some suggestions are made to explain the results obtained.

REFERENCES

(1) Adams, B. A., and Holmes, E. L. Adsorptive Properties of Synthetic Resins. *J. Soc. Chem. Ind.* (London), **54**: 1. 1935.

(2) Almy, E. F., and Garrett, O. F. Preparation of High Grade Crude Lactose. U. S. Patent 2,477,558. 1949.

(3) American Public Health Association. *Standard Methods for the Examination of Water and Sewage.* 9th ed., p. 23. Publ. jointly by the Am. Public Health Assoc. and Am. Water Works Assoc. New York, N. Y. 1946.

(4) Association of Official Agricultural Chemists. *Official and Tentative Methods of Analysis.* 6th ed., p. 337. Assoc. of Offic. Agr. Chemists. Washington 4, D. C. 1945.

(5) Barber, H. H., and Koltoff, I. M. A Specific Reagent for the Rapid Gravimetric Determination of Sodium. *J. Am. Chem. Soc.*, **50**: 1625. 1928.

(6) Boyd, G. E. Ion Exchange. *Ann. Rev. Phys. Chem.*, **2**: 309. 1951.

(7) Bray, R. H. A Test for Replaceable and Water Soluble Potassium in Soils. *J. Am. Soc. Agron.*, **24**: 312. 1932.

(8) Burgess Zeolite Co., Ltd. Reducing Calcium Content in a Liquid Milk Product. British Patent 542,846. 1942.

(9) Christiansen, G., Jenness, R., and Coulter, S. T. Estimation of Calcium Ion Activity in Milk and Other Biological Fluids. *J. Dairy Sci.*, **34**: 488. 1952.

(10) Chrystler, L. H., and Almy, E. F. Preparation of a Stabilized Cream Product. U. S. Patent 2,503,866. 1950.

(11) Fiske, C. H., and Subbarow, Y. Colorimetric Determination of Phosphorus. *J. Biol. Chem.*, **66**: 375. 1925.

(12) Gehrke, C. W., and Almy, E. F. Action of Mineral Ion Exchange Resins on Milk Constituents. *Ind. Eng. Chem.*, **42**: 2344. 1950.

(13) Haller, H. S., and Bell, B. W. Ion Exchange as a Means of Improving the Keeping Quality of Frozen Homogenized Milk. *J. Dairy Sci.*, **33**: 406. 1950.

(14) Haller, H. S., and Morin, A. O. Ion Exchange as a Means of Varying Salt Constituent of Milk. *J. Dairy Sci.*, **33**: 395. 1950.

(15) Hetrick, J., and Whitney, R. M. Determination of Nitrogen in Milk by Direct Nesslerization of the Digested Sample. *J. Dairy Sci.*, **32**: 111. 1949.

(16) Josephson, D. V., and Reeves, C. B. Utilization of Mineral Ion Exchange Principle in Stabilizing Evaporated Milk. *J. Dairy Sci.*, **30**: 737. 1947.

(17) Kolthoff, I. M., and Sandell, E. F. *Quantitative Inorganic Analysis.* Rev. ed., p. 340. Macmillan Co., N. Y. 1947.

(18) Lampitt, L. H., and Rooke, H. S. Citric Acid in Milk and Its Determination. *The Analyst*, **61**: 654. 1936.

(19) Lyman, J. F., Browne, E. H., and Otting, H. E. Readjustment of Salts in Milk by Base Exchange Treatments. *Ind Eng. Chem.*, **25**: 1297. 1933.

(20) McGlasson, E. D., and Boyd, J. C. Improved Methods in the Recovery of Lactose Using Ion Exchange Resin. *J. Dairy Sci.*, **34**: 119. 1951.

(21) Meade, R. E., and Clary, P. D., Jr. Methods of Making Novel Products from Whey. U. S. Patent 2,465,906. 1949.

(22) Meade, R. E., and Clary, P. D., Jr. Methods of Making Lacteal Food Products. U. S. Patent 2,465,907. 1949.

(23) Myers, R. J. Replacing Cations in Milk. U. S. Patent 2,511,825. 1950.

(24) Otting, H. E. Stabilizing Evaporated Milk. U. S. Patent 2,490,599. 1949.

(25) OVERMAN, O. R., KEIRS, R. J., AND CRAINE, E. M. Composition of Herd Milk of the Brown Swiss Breed. Ill. Agr. Expt. Sta., *Bull.* 567. 1953.

(26) ROWLAND, S. J. Determination of Nitrogen Distribution in Milk. *J. Dairy Research*, 9: 42. 1938.

(27) THOMPSON, H. S. On the Absorbent Power of Soils. *J. Roy. Agr. Soc., England*, 11: 68. 1850.

(28) UNDERWOOD, G. E., AND DEATHERAGE, F. E. Use of an Ion Exchange Resin for the Hydrolysis of Casein and Coffee Proteins. *Science*, 115: 95. 1952.

(29) VERMA, I. S. A Study of the State of Solution of the Naturally Occurring Salts in Milk. Ph.D. thesis, Univ. of Wisconsin. 1950.

(30) WAY, J. T. On the Power of Soils to Absorb Manure. *J. Roy. Agr. Soc., England*, 11: 313. 1850; 13: 123. 1852.

(31) WILDASIN, H. L., AN DOAN, F. J. Some Additional Influences Affecting the Stability of Cows' Milk in the Frozen State. *J. Dairy Sci.*, 34: 438. 1951.

STUDIES ON THE USE OF PERMANENT MILK PIPELINES IN DAIRY BARNS. III. LABOR (TIME) REQUIREMENTS FOR OPERATION OF PIPELINE AND CONVENTIONAL INSTALLATIONS IN A STANCHION BARN

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Permanent milk pipelines have been introduced into the dairy industry as a means of increasing operating efficiency and reducing costs without impairing milk quality. It has been demonstrated that milk lines in manufacturing plants *(4, 6)* and on farms *(1, 2)* can be cleaned effectively in position and that certain economies result from in-position cleaning in processing plants *(5)*. Although processors are concerned primarily with labor economy involved in cleaning milk lines in position vs. disassembled, the problem is more complex at the farm level. In addition to disassembly vs. in-position cleaning, the relative labor economy of the pipeline and conventional systems for milking and milk handling must be considered.

This report presents the results obtained in a series of studies designed to measure the labor (time) requirements for the operation of pipeline and conventional installations in a stanchion dairy barn.

EXPERIMENTAL PROCEDURE

General. The labor (time) expended in milking a herd of 24 cows with the pipeline and three conventional systems, two basic and one modified, was measured essentially by the method described by Barnes *(3)* using a stop watch calibrated in one-hundredths of a minute. The operations studied were premilking, milking, and clean-up procedures.

Facilities and equipment. A glass pipeline, 200 ft. in length, was arranged in a complete circuit over two 12-cow stanchion lines in a barn in which the cows faced outward. Each stanchion line was provided with an overhead track which supported a movable dairy scale from which the milking machine assembly was suspended. The stanchion lines were serviced by stall cocks located midway between the individual members of each pair of cows. The two lines of cows were milked simultaneously by one machine on each line. The machines operated at 48 pulsations per minute and vacuum equivalent to 15 in. of mercury.

The conventional machine milking manual-carry-away systems involved the use of two or four complete milking units. In order to accelerate milking operations, an extra milker pail was provided when the conventional systems were used. Depending upon the number of units used in the conventional system, the two stanchion lines of cows were milked simultaneously with either one or two machines per line.

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In all trials the rinse solutions, strip cup, and udder wash cloths were on an equipment cart which was moved when necessary by the operator.

Operations. When the pipeline was used, the premilking operations included: *(a)* rinsing the pipeline, milk valves, and cold wall tank with a chlorine solution (200 p.p.m.), *(b)* positioning the strainer and assembling the milk line from the surge chamber to the cold wall tank, *(c)* assembling the milker units, and *(d)* preparing the rinse solutions, udder wash cloths, strip cup, and sample bottles.

Premilking operations in the conventional systems included only items *c* and *d* listed above with the exception that pails for carrying the milk were placed on the cart and the sample bottles were left in the milk room. Premilking operations were considered complete when all equipment was in position and ready for use in the barn.

In the pipeline operations, the milk was drawn into the suspended milker pail to be weighed, sampled when necessary, and carried by vacuum through the pipeline to a surge chamber from which it was pumped through an in-line strainer into a cold wall tank for cooling and storage.

When the conventional systems of milking were used, the milk was poured from the machine to pails and carried to the milk house, where it was weighed, sampled as necessary, and strained into the cold wall tank.

Clean-up operations consisted of rinsing, washing, sanitizing, and storing the milk-bearing equipment in accordance with standard dairy practices. The pipeline was cleaned in position with chlorine solution (200 p.p.m.) as the germicidal rinse. Details of the clean-up operations have been reported previously *(2)*.

Work performed. One man performed the work involved in milking with the pipeline system. The work included: *(a)* preparing cows by washing the udder and teats and using a strip cup on each quarter, *(b)* rinsing the teat cups in water and in a germicidal solution after each cow was milked, *(c)* connecting milker unit to vacuum line and pipeline, *(d)* placing the teat cups on the cow, *(e)* machine stripping, *(f)* weighing, sampling, and releasing the milk into the line, *(g)* recording milk yield, and *(h)* moving equipment cart and milker units when necessary.

Two men performed the work involved in milking the herd with the conventional system. The operator's work was essentially that described above with the exceptions that pipeline operations were eliminated and the operator was required to change the milker head to the extra pail. The second man poured the milk into pails and carried it to the milk house, where it was weighed, sampled, and strained, and the weight was recorded. The milk from each cow was removed immediately after she was milked.

The premilking and clean-up work in all systems of milking was performed by the operator who did the milking.

Work pattern. The milking procedures employed were those commonly used in dairy farm operations. The experienced dairyman who performed this work established the milking routine in preliminary trials and followed it throughout the investigation. No attempt was made to increase efficiency by changing the

established procedure during the major portion of the study. In a series of later experiments with the four-unit conventional system, the work pattern was modified to the extent that the helper prepared the cows and used the strip cup during the time he was not engaged in carrying milk. In addition, the milk from the two cows was carried simultaneously to the milk room. Data pertaining to the modified work pattern are presented in the tables under the heading of "Four Unit, Conventional, Modified."

The work pattern was essentially the same for the systems studied. The operator kept the milking machines in a juxtaposition across the alleyway during milking. At the start of milking operations, the first cow in one stanchion line was prepared and the teat cups were attached, after which the same procedure was followed with the first cow in the opposite stanchion line. The cows adjacent to the machines were prepared before those being milked were machine-stripped and before the machines were moved. This procedure was followed until the herd was milked.

When the four-unit conventional system was used, after the initial cycle, it was necessary for the operator to prepare two cows in each stanchion line before machine stripping the four cows being milked.

With both conventional systems the operator changed the milker unit to the extra pail immediately after each cow was milked. This prevented unnecessary delays in his work if the helper was not present to pour the milk into the carrying pails.

Cows. The cows were not selected or arranged for stage of lactation, level of production, or ease and rapidity of milking but were left in the order established by normal herd operations. Consequently, production ranged from 8 to 34 lb. and averaged 17.7 lb. of milk per cow per milking.

The number of cows in the stanchion lines was limited to 24. Because some animals had to be taken out of the herd in accordance with accepted dairy farming practices, the numbers of animals on experiment at various times were 20, 22, or 24. The position of the individual animals in the stanchion lines remained relatively constant.

The data are calculated on the basis of a 24-cow herd and are expressed as minutes and decimal portions thereof.

RESULTS AND DISCUSSION

The time values obtained when the experimental systems of milking were applied to a herd of 20 to 24 cows are summarized in Table 1. More clock hours were required for pipeline operations than when the herd was milked by use of the conventional systems. Pipeline operations required approximately 73 minutes of clock time for the milking of 24 cows, whereas the two-unit, four-unit, and modified conventional systems consumed approximately 63, 51, and 42 minutes, respectively.

On the basis of total man-minutes spent milking 24 cows, the pipeline system had a lower labor (time) requirement than did the conventional systems. The two-unit, four-unit, and the modified four-unit conventional systems

TABLE 1

Elapsed time values for milking operation using different systems

System of milking	No. of cows in herd	Operator time	Helper time	Total man-min. expended	Man-min. per cow	Calculat. man-min. per 24 cows	Mean man-min.	
							per cow	per herd
		(Min.)	*(Min.)*					
	24	75.68	0	75.68	3.15	75.68	....	....
	24	75.85	0	75.85	3.16	75.85	....	....
	20	60.90	0	60.90	3.05	73.08	....	....
Two-unit,	20	56.00	0	56.00	2.80	67.20	....	....
pipeline	20	59.34	0	59.34	2.97	71.21	....	....
	20	61.20	0	61.20	3.06	73.44	....	....
	20	60.10	0	60.10	3.01	72.12	....	....
	20	60.90	0	60.90	3.05	73.08	3.03	72.71
	24	63.85	59.49	123.34	5.14	123.34	....	....
	24	62.60	56.44	119.04	4.96	119.04	....	....
Two-unit,	22	60.00	53.36	113.36	5.15	123.65	....	....
conventional	22	69.65	53.65	113.30	5.15	123.57	....	....
	22	60.15	53.15	113.30	5.15	123.57	....	....
	22	57.78	51.75	109.53	4.92	119.47	5.08	122.11
	24	48.04	39.38	87.42	3.64	87.42	....	....
	24	53.09	45.62	98.71	4.11	98.71	....	....
Four-unit,	24	47.58	42.45	90.03	3.75	90.03	....	....
conventional	24	51.20	45.97	97.17	4.05	97.17	....	....
	24	56.11	50.00	106.11	4.42	106.11	....	....
	24	49.38	44.37	93.75	3.91	93.75	3.98	95.53
Four-unit,	24	40.65	41.67	82.32	3.43	82.32	....	....
conventional,	24	41.17	42.00	83.17	3.46	83.17	....	....
modified	24	42.67	43.65	86.32	3.60	86.32	3.50	83.93

required 122, 96, and 84 man-minutes, respectively, whereas an operation time of 73 man-minutes was obtained by use of the pipeline. The average man-minutes per cow for the above systems, in the order listed, were 5.03, 3.98, 3.50, and 3.03.

The general work pattern, the distribution of work, and the time spent performing the individual steps in the milking operations are shown in Table 2. It should be pointed out that the data contained in the last column of Table 2 were obtained 6 months after the completion of the other experiments. However, since experiments using the pipeline as a control showed that the mean time value of three milking operations of 24 cows each was within 1 minute of those values obtained in the earlier pipeline experiments, the time values for the modified conventional four-unit system were calculated and included in Tables 1 and 2. The changed helper work pattern used in the modified system necessitated a change in analytical procedure. Consequently, the helper data on this system have been calculated only as time spent in preparing cows, delivering milk, and idle time.

Analysis of data in Tables 1 and 2 indicates that the milking machine operator worked at a relatively high rate of efficiency regardless of the system employed. The proportion of the average elapsed time per cow (Table 1) accounted for by the work units listed in Table 2 was approximately 88, 89,

92, and 86% for the pipeline, two-unit, four-unit, and four-unit modified conventional systems, respectively. Conversely, the helper's time was not utilized efficiently in the conventional systems because of periods of idleness inherent in these systems. Thus, only approximately 46, 53, and 55% of the helper's time (Table 1) was accounted for by the work units listed in Table 2 when the above-listed conventional systems were studied. On the basis of total man-

TABLE 2

Time required to perform individual steps in milking operation using different systems

Work accomplished	Mean operation time[a]			
	2-unit pipeline	2-unit conventional	4-unit conventional	4-unit[a] conv. modified
A (Operator)		*(minutes per cow)*		
Prepare cows	0.44	0.42	0.48	.....
Rinse cups, turn on vacuum	0.13	0.20	0.18	0.18
Place cups on cows	0.18	0.21	0.22	0.19
Machine strip	1.08	1.30	0.88	0.90
Weigh and sample	0.22	.....	.....	.....
Release milk to line	0.27	.....	.....	.....
Record weight	0.13	.....	.....	.....
Move equipment cart	0.12	0.06	0.03	0.06
Move milker units	0.09	.....	.....	.....
Replace head on pail	.....	0.18	0.17	0.16
TOTAL	2.66	2.37	1.96	1.49
B (Helper)				
Prepare cows	.....	.....	.....	0.33
Pour milk	.....	0.17	0.17	.....
Carry milk	.....	0.19	0.12	0.60[b]
Weigh and sample	.....	0.34	0.38	.....
Strain milk	.....	0.11	0.10	.....
Return from milk room	.....	0.23	0.15	.....
Idle	.....	1.30	0.88	0.80
TOTAL	.....	2.34	1.80	1.73
TOTAL A plus B	2.66	4.71	3.76	3.22

[a] Mean of two trials of 24 observations each.
[b] Includes pour, carry, weigh and sample, strain milk and return from milk room.

minutes expended (Table 1) versus those utilized doing work units listed in Table 2, the relative over-all efficiency of the pipeline, two-unit, four-unit, and the modified four-unit systems was approximately 88, 69, 74, and 70%, respectively.

Although machine operator efficiency appeared to be at a high level during these studies, the work data in Tables 1 and 2 may be misleading if the time the milking machines were on the cows is not considered. It was observed in some instances that the time the machine was on a particular cow was related to the time required for the simultaneous milking of other cows. Thus, one slow milker in a group could increase the machine time on another cow when two units were used, or on three other cows when four units were operated. During the trials represented in Table 2 the milking machines were on for an average of 5.0 (range 3.5 to 7.5) and 4.4 (range 3.3 to 6.2) minutes per cow for the pipeline and two-unit conventional systems, respectively. An average

machine-time per cow of 7.0 minutes (range 5.0 to 9.1) was recorded for the four-unit conventional system. The latter machine-time is too long to be consistent with accepted dairy production practices. Since the cows milked out in less than 5 minutes machine-time with the two-unit pipeline and conventional systems, it is evident that the work load and the arrangement thereof was the key factor contributing to the excessive machine-time in the four-unit conventional system. By changing the work distribution, the average machine-time per cow was reduced from 7.0 minutes to 5.8 minutes (range 2.9 to 9.2) when the modified four-unit system was used.

A comparison of the time values for premilking, milking, and clean-up operations for the four experimental systems of milking is presented in Table 3. The economy of man-minutes realized by using the pipeline system during milking operations was somewhat offset by the time requirements for premilking and clean-up operations. Even so, less total man-minutes were required for the over-all work with the pipeline system than with the two-unit and four-unit conventional systems. However, as shown in Table 3, the time expended

TABLE 3

Time required for each operation in the milking of 24 cows using different systems

Operation	Total time			
	Pipeline	2-unit	4-unit	4-unit, modified
	(man-minutes)			
Premilking[a]	16.21	7.68	11.09	11.09[b]
Milking	72.71	122.11	95.53	83.93
Clean up[a]	20.73	8.73	11.76	11.76[b]
Total	109.65	138.52	118.38	106.78
Ave. per Cow	4.56	5.77	4.93	4.45

[a] Mean of three observations.
[b] Data from study of four-unit conventional system.

using the modified four-unit system was equivalent to or less than that for the pipeline system. In addition, the modified four-unit system work was completed in approximately 65 minutes of clock time as compared to a total of 109 minutes of clock time expended accomplishing the work involved in the pipeline system of milking.

It was observed that one operator expended 73 minutes milking 24 cows by the pipeline system, whereas a second and equally experienced dairyman required an average of 84 minutes to accomplish the identical work load with the same cows. This emphasizes the fact that the time requirements for milking operations are subject to the work habits of the operator, as well as to work layout and the habits of the cows being milked. Consequently, the data presented herein are considered as relative rather than absolute values.

SUMMARY

The relative labor (time) requirements of a pipeline and conventional milking systems used in a 24-cow stanchion barn have been assessed. Premilking,

milking, and clean-up operations in the pipeline system required more clock hours than did the conventional systems. However, the data show that more efficient utilization of labor (man-minutes) during the milking operation occurred when the pipeline system was used, as compared to the two- and four-unit conventional system. A modified four-unit two-man conventional system and the two-unit one-man pipeline system had approximately equal labor requirements (man-minutes) for accomplishing the premilking, milking, and clean-up operations.

REFERENCES

(1) ALEXANDER, M. H., NELSON, W. O., AND ORMISTON, E. E. Studies on the Use of Permanent Milk Pipelines in Dairy Barns. I. Effect of Different Types of Installation on Milk Quality. *J. Dairy Sci.*, **35**: 874. 1952.

(2) ALEXANDER, M. H., NELSON, W. O., AND ORMISTON, E. E. Studies on the Use of Permanent Milk Pipelines in Dairy Barns. II. Milk Quality as Affected by Types of Installations and Sanitation Procedures. *J. Dairy Sci.*, **36**: 303. 1953.

(3) BARNES, R. M. *Motion and Time Study.* 3rd ed. John Wiley and Sons, Inc., New York. 1949.

(4) FISKER, A. N. Chemical Cleaning of the Pasteurization Plant. *XIIth Intern. Dairy Congr. Proc.*, **3** (3): 319. 1949.

(5) FLEISCHMAN, F. F., JR., AND HOLLAND, R. F. Permanent Pipelines Cut Cleaning Costs. *Food Eng.*, **23** (11): 58. 1951.

(6) POSTHUMUS, G. Cleaning of Dairy Equipment and Control of the Efficiency of Cleaning Process in Holland. *XIIth Intern. Dairy Congr. Proc.*, **3** (3): 288. 1949.

REPORT OF COMMITTEE ON BUTTER[1]

American Dairy Science Association

It is the committee's belief that its function should center primarily on sponsoring activities that have for their purpose the improvement and standardization of the quality of butter as sold to the ultimate consumer. The butter industry should sponsor a vigorous, continuing research program involving the manufacture, storage, and merchandising of butter. The cooperation of equipment manufacturers, experiment stations, creameries, and merchandisers is necessary. An industry that manufactures and distributes 1.4 billion pounds butter per year must not stand still.

The following 4-point program is recommended:

I. Improvement of butter quality

A. *Object*

The butter manufactured should be brought into the highest possible grade. The creameries should manufacture butter of uniform composition, high quality, attractive color, and excellent keeping quality.

B. *Method of procedure*

1. All states, individually, should establish laws and/or regulations governing minimum sanitary requirements for producing and handling milk and cream, avoiding variations of regulations in the general geographical areas. This should include suitable standards for sediment content as well as standards for general quality.
2. The industry should sponsor compulsory payment for milk and cream on the basis of quality. The grades and price differentials should be such that they will favor the grade of milk or cream that will be suitable for the manufacture of the higher grades of butter. All milk or cream, no matter what grade, should be transported in sanitary, well-protected containers to the creamery.
3. Milk and cream quality improvement educational programs should be intensified. They should be a part of the dairy extension programs of each agricultural college and of the state dairy producers' and butter manufacturers' associations.
4. Where butter is manufactured in sizable amounts the state agricultural college should offer a butter scoring and analysis service to the

[1] The Executive Board of the American Dairy Science Association has accepted the report of the committee on butter of the Manufacturing Section as a document which merits the serious consideration of all those who are striving to improve the quality and acceptability of butter.

creameries. Information regarding the correction of defects in quality should be reported to the buttermakers. To be successful, industry cooperation and perhaps state plus industry financing may be necessary. Cooperation with the state departments of agriculture is desirable. Reports should be kept confidential to the extent that the origin of the samples be withheld from public knowledge.

5. Agricultural colleges in states where butter is an important manufactured product should offer short courses in butter manufacture.
6. Certificates of proficiency should be granted to buttermakers whose ability to make butter can be proved both by actual manufacture and by oral and written examination.
7. States that do not have a creamery inspection system should adopt one. This should include inspection of condition of building and surroundings, rodent and insect control, physical condition of equipment, sanitation, sewage disposal, and water supply.

II. Tests for quality

Further improvement and simplification of tests for determining the quality of milk, cream, and butter are desirable. As the demand for butter of higher grade increases, the several quality tests that are now available should be refined and more widely used. For example, tests for keeping quality, pH, yeasts and molds, coliform bacteria, extraneous matter, metal contamination, enzymes (lipase), lipolytic and proteolytic bacteria, and water insoluble and butyric acids, should be more commonly used for checking the quality of pasteurized cream and butter.

III. Manufacturing, packaging, storing, distributing butter

A. *Object*

The object should be to develop methods for procurement of milk and cream and for manufacture and distribution so that butter of excellent quality can be economically manufactured and offered to the consumer at prices as favorable as possible.

B. *Method of procedure*

1. The problems of the butter industry should be determined; then funds for a long-range research and development program should be obtained. Experiment stations that have the facilities should test new methods of manufacture and test new equipment.
2. Butter may deteriorate during storage as the result of *(a)* the growth of microorganisms, *(b)* enzyme activity, *(c)* action of chemical catalyzers, and *(d)* exposure to odors. Research is necessary to find methods that are most satisfactory for manufacturing, packaging,

and storing so that the butter will retain its desirable flavor from the time it leaves the churn until it reaches the consumer.

3. There also is a need for experimentation for the purpose of further improvement of the body, texture, and spreadability of butter and, more than anything else, for work with the buttermakers to apply improved techniques.
4. Consumer tests might be made to determine the type of butter most desired by the public.
5. The utilization of buttermilk should be fully investigated.

IV. Standardization of the color of butter

It is recommended that the standardization of the color of butter by general trade area be sponsored by the butter industry in cooperation with agricultural colleges.

Committee on butter:

D. L. Fourt, Idaho
C. A. Iverson, Iowa
Chris Jensen, North Dakota
N. E. Fabricius, Wisconsin
W. L. Slatter, Ohio
G. H. Wilster, Oregon, *Chairman*

PROGRAM

FORTY-NINTH ANNUAL MEETING

of the

AMERICAN DAIRY SCIENCE ASSOCIATION

THE PENNSYLVANIA STATE UNIVERSITY

STATE COLLEGE, PENNSYLVANIA

JUNE 20-24, 1954

Program Committee

H. B. HENDERSON, Georgia, *Chairman*

S. N. GAUNT, Massachusetts — C. R. GEARHART, Pennsylvania

A. J. MORRIS, Utah — P. L. KELLY, Nebraska

GENERAL PROGRAM

Sunday, June 20

9:00 A.M. on — **Meeting of the Executive Board,** *Room 205 B, Old Main*

Monday, June 21

9:00 A.M. on — **Meeting of the Executive Board,** *Room 205 B, Old Main*

10:00 A.M. on — **Registration,** *West Dorms Lounge*

2:00, 3:00, and 4:00 P.M. — **Buses leave for tour of Dairy Production Center**

Parking area north of registration headquarters

1:00–5:00 P.M. — Buses circulate all afternoon from *Dairy Building* to Special Grassland Field Day features and return.

8:00 P.M. — **"Howdy Nite,"** *Recreation Hall*

8:00 P.M. — D.H.I.A. — I.B.M. Committee — Round table discussion on I.B.M. (open to all), *Room 10, Sparks Building*

Tuesday, June 22

8:00 A.M. on — **Registration,** *West Dorms Lounge*

9:45 A.M. — **Opening Session,** *Schwab Auditorium*

Dr. D. V. Josephson, *Head, Department of Dairy Husbandry, The Pennsylvania State University, Presiding.*

PRELUDE

Professor G. E. Ceiga, *Organist, Department of Music, The Pennsylvania State University.*

NATIONAL ANTHEM
Leader — Dr. Henry S. Brunner, *Department of Agricultural Education, The Pennsylvania State University.*

INVOCATION
Professor J. H. Frizzell, *Chaplain Emeritus, The Pennsylvania State University.*

WELCOME ADDRESS
Dr. Lyman E. Jackson, *Dean, College of Agriculture, The Pennsylvania State University.*

PRESIDENTIAL ADDRESS
Dr. Walter V. Price, *University of Wisconsin.*

INTRODUCTORY REMARKS
Dr. Milton Eisenhower, *President, The Pennsylvania State University.*

ADDRESS
The Honorable Ezra Taft Benson, *Secretary of Agriculture, United States Department of Agriculture.*

POSTLUDE
Professor G. E. Ceiga.

Section Meetings

1:30–5:00 P.M. MANUFACTURING SECTION A
Symposium: Milk Proteins
Room 119, Osmond Laboratory

MANUFACTURING SECTION B
1:30–3:00 P.M. 1. Methods for Increasing Productivity in Dairy Plants
3:10–5:00 P.M. 2. In-Plant Training of Supervisory Personnel
Room 109, Agricultural Building

1:30–5:00 P.M. PRODUCTION SECTION A
Artificial Insemination and Reproduction
Room 10, Sparks Building

1:30–5:00 P.M. PRODUCTION SECTION B
Calf Nutrition
Room 121, Sparks Building

1:30–4:30 P.M. EXTENSION SECTION
Opening Business Session, Teaching Methods, and Exhibits
Room 316, Sparks Building

7:30 P.M. **Officers' Reception** (Informal)
Recreation Hall

Wednesday, June 23

Section Meetings

8:00–10:30 A.M. MANUFACTURING SECTION A
Microbiology
Room 109, Agricultural Building

8:00–10:30 A.M. MANUFACTURING SECTION B
Cheese
Room 119, Osmond Laboratory

10:30–11:00 A.M. COMBINED MANUFACTURING SECTIONS A AND B
Room 109, Agricultural Building

8:30–11:00 A.M. PRODUCTION SECTION A
Silage Production and Feeding
Room 10, Sparks Building

8:30–11:00 A.M. PRODUCTION SECTION B
Physiology
Room 121, Sparks Building

9:00–11:00 A.M. EXTENSION SECTION
Use of Herd Improvement Associations to Improve Dairying
Room 316, Sparks Building

11:00 A.M.–NOON MANUFACTURING SECTION BUSINESS MEETING
Room 109, Agricultural Building

11:00 A.M.–NOON PRODUCTION SECTION BUSINESS MEETING
Room 121, Sparks Building

11:00 A.M.–NOON EXTENSION SECTION BUSINESS MEETING
Room 316, Sparks Building

1:30–4:30 P.M. MANUFACTURING SECTION A
Market Milk and Ice Cream
Room 109, Agricultural Building

1:30–4:30 P.M. MANUFACTURING SECTION B
Dairy Chemistry
Room 119, Osmond Laboratory

4:30 P.M. MANUFACTURING SECTION BUSINESS MEETING
Room 109, Agricultural Building

1:10–3:20 P.M. PRODUCTION SECTION A
Rumen Physiology
Room 10, Sparks Building

1:30–3:20 P.M. **Joint Session of Production Section B and Extension Section**
Sire Evaluation
Room 121, Sparks Building

3:30–4:30 P.M. **Joint Session of Production and Extension Sections**
Committee Reports:
Report of National Association of Artificial Breeders
Report of Purebred Dairy Cattle Association
Room 121, Sparks Building

7:15 P.M. **Recognition Program and Entertainment**
Presentation of Honor Awards and Installation of Officers
Schwab Auditorium

Thursday, June 24

Section Meetings

8:30–10:30 A.M. MANUFACTURING SECTION A
Milk Fats
Room 119, Osmond Laboratory

8:30–10:30 A.M. MANUFACTURING SECTION B
Concentrated Milks
Room 109, Agricultural Building

8:30–10:30 A.M. PRODUCTION SECTION A
Pastures and Grazing
Room 10, Sparks Building

8:30–10:30 A.M. PRODUCTION SECTION B
Genetics
Room 121, Sparks Building

9:00–10:30 A.M. EXTENSION SECTION
4-H Club Work
Room 316, Sparks Building

10:30 A.M.–NOON GENERAL ASSOCIATION BUSINESS MEETING
Room 121, Sparks Building

Section Meetings

1:30–4:30 P.M. MANUFACTURING SECTION
Symposium — Dairy Waste
Room 119, Osmond Laboratory

1:30–4:30 P.M. PRODUCTION SECTION A
Physiology
Room 10, Sparks Building

1:30–4:30 P.M. PRODUCTION SECTION B
Nutrition and Management
Room 121, Sparks Building

1:30–4:00 P.M. EXTENSION SECTION
Dairy Cattle Health and Forage Evaluation
Panel Discussion and Forum
Room 316, Sparks Building

7:30 P.M. ENTERTAINMENT
Room 10, Sparks Building

ENTERTAINMENT PROGRAM

Entertainment for Men and Women

Mon., June 21 8:00 P.M.—"HOWDY NITE"
Recreation Hall

Tues., June 22 7:30 P.M.—OFFICERS' RECEPTION AND DANCE (Informal)
Recreation Hall

Wed., June 23 7:15 P.M.—RECOGNITION PROGRAM AND ENTERTAINMENT
Schwab Auditorium

Thurs., June 24 7:30 P.M.—MOVIE: "Here You Are" by Bill Bryant, Johnson and Johnson
Color Slides: Pennsylvania Agriculture, by George Johnson, The Pennsylvania State University
Room 10, Sparks Building

Entertainment for Women

(see separate program)

Tues., June 22 10:00 A.M.—KAFFISCHTURN (coffee hour)
Simmons Hall Lounge

1:00 P.M.—TRIP TO PENN'S CAVE (no children)

Wed., June 23 9:00 A.M.—TRIP TO PENN'S CAVE (mothers, and children over 10)

9:30 A.M.—"WALKY-TALKY" TOURS

1:00 P.M.—OLD BOALSBURG TOUR

Thurs., June 24 9:30 A.M.—"WALKY-TALKY" TOURS

2:30 P.M.—TEA
Simmons Hall Lounge

Children's Program

(see separate program)

NURSERY SCHOOL (age 3 through 5)

Tues., June 22
Wed., June 23
Thurs., June 24
9:00 A.M. to NOON and 3:00 to 5:00 P.M.,
University Nursery School, Home Economics Building

INTERMEDIATE (age 6 through 9)

Tues., June 22 9:30 A.M. to 5:00 P.M., Whipple's Dam, day camp activities

7:00 to 8:30 P.M., Movies and Music
Room 10, Sparks Building

Wed., June 23 9:00 A.M. to NOON, Games, treasure hunt, and treat

12:45 to 5:00 P.M., Movies, M.I. Museum, Dairy Barns, Horse Show

7:00 to 8:30 P.M., Movies and magic
Room 10, Sparks Building

Thurs., June 24 9:00 A.M. to NOON, Games and Crafts, *Sunset Park*

2:30 to 5:00 P.M., Swimming, Glennland Pool

SENIORS (age 10 and over)

Tues., June 22 9:30 A.M. to 5:00 P.M., Whipple's Dam, day camp activities

7:00 to 8:30 P.M., Movies and music
Room 10, Sparks Building

Wed., June 23 9:00 A.M. to NOON, Trip to Penn's Cave (if accompanied by parent)
or
Organized play, *Sunset Park*

12:45 to 5:00 P.M., Movies, M.I. Museum, Dairy Barns, Horse Show

7:00 to 8:30 P.M., Movies and magic, *Room 10, Sparks Building*

8:30 to 11:30 P.M., Dancing, *Fairmont Park*, as guests of State College Youth Club (12 yr. and up)

Thurs., June 24 9:00 A.M. to NOON, Roller Skating, *Evergreens Coliseum*

2:30 to 5:00 P.M., Swimming, Glennland Pool

MANUFACTURING SECTION

Tuesday, June 22

WILLIAM M. ROBERTS, *Presiding*

1:30–5:00 P.M.

Symposium: Milk Proteins.
ARTHUR M. SWANSON, *Chairman*
Room 119, Osmond Laboratory

Separation and fractionation of milk proteins. T. L. McMeekin, *Eastern Utilization Research Branch, U.S.D.A.*

Sizes of the casein complex particles in milk. T. F. Ford, *Washington Utilization Research Branch, U.S.D.A.*

The effects of heat on the milk serum proteins. Robert Jenness, *University of Minnesota.*

Contributed Papers:

M1 Physico-chemical changes induced in casein by *Streptococcus agalactiae.* G. W. Malaney, H. H. Weiser, and Q. Van Winkle, *The Ohio State University.*

M2 Heat denaturation of the specific serum proteins in milk. B. L. Larson and G. D. Rolleri, *University of Illinois.*

M3 The effect of heat and salts on the centrifugal sedimentation rates of milk proteins. L. F. Edmondson and N. P. Tarassuk, *University of California.*

M4 Sedimentation of milk proteins as a function of certain ions. S. J. Bishov and J. H. Mitchell, Jr., *Food and Container Institute for the Armed Forces, Chicago.*

M5 The activity of calcium chloride, citrate and dihydrogen phosphate in casein sols. H. E. Affsprung, C. W. Gehrke, and J. M. Baker, *University of Missouri.*

Practical Panels:

G. H. HARTMAN, *Presiding*
Room 109, Agricultural Building

1:30–3:00 P.M.

Modern methods for increasing productivity in dairy plants.
Chairman: David Burrell, *Cherry-Burrell, Inc., Chicago.*

Panel members:
Harold Dunlap, *H. P. Hood & Sons, Boston*
A. E. Geiss, *Bowman Dairy Company, Chicago*

3:10–5:00 P.M.

In-plant training of supervisory personnel.
Chairman: Ray Tardiff, *Breyer's Ice Cream Co., Philadelphia*

Panel members:
Ansel Estell, *Borden Company, Columbus, Ohio*
Ralph Traxler, *Kraft Cheese Company, Chicago*

Wednesday, June 23

8:00–10:30 A.M.

Section A. **Microbiology.**
W. M. ROBERTS, *Presiding*
Room 109, Agricultural Building

M6 Characteristics of some lactobacilli found in milk. J. E. Edmondson and R. G. Jensen, *University of Missouri.*

M7 Stimulation of lactic acid bacteria by substances concomitant to certain proteolytic enzymes. D. E. Kizer, L. Hankin, M. L. Speck, and L. W. Aurand, *North Carolina State College.*

M8 Metabolism of amino acids by *Lactobacillus casei.* T. Kristoffersen and F. E. Nelson, *Iowa State College.*

M9 Studies on corn steep liquor in the nutrition of lactic acid bacteria. H. E. Kennedy, M. L. Speck, and L. W. Aurand, *North Carolina State College.*

M10 Acetate-citrate metabolism of *Streptococcus lactis* and *Streptococcus cremoris.* D. E. Kizer and M. L. Speck, *North Carolina State College.*

M11 Changes in the bacterial population of milk due to the addition of some chemical bactericides. F. J. Babel, *Purdue University.*

M12 Thermal death time studies of *Brucella abortus* in milk. F. R. Kronenwett, S. A. Lear, and H. J. Metzger, *Rutgers University.*

M13 The effect of some factors on the growth of "psychrophilic" bacteria. W. C. Lawton and F. E. Nelson, *Iowa State College.*

M14 A study of coliforms isolated from pasteurized milk. W. G. Walter and L. T. Christian, *Montana State College.*

M15 Bacteriological studies of milk from menadione fed cows. H. H. Wilkowske, W. A. Krienke, L. R. Arrington, and E. L. Fouts, *University of Florida.*

8:00–10:30 A.M.

Section B. **Cheese.**

A. J. MORRIS, *Presiding*

Room 119, Osmond Laboratory

M16 The use of a lyophilized lactic starter in the preparation of cottage cheese and cultured milk. J. C. Watts and H. E. Calbert, *University of Wisconsin.*

M17 A study of some factors affecting the quality of cottage cheese. M. P. Rhodes and L. G. Harmon, *Texas Technological College.*

M18 The effect of fortification of fluid skimmilk with nonfat dry milk solids on the yield of cottage cheese curd. R. W. Mykleby and B. M. Zakariasen, *Land O' Lakes Creameries, Inc., Minneapolis.*

M19 Influence of the bulk handling of raw milk on its rennet coagulation time. Harold Rapp and H. E. Calbert, *University of Wisconsin.*

M20 Flavor development in pasteurized milk Cheddar cheese. The effect of inoculating milk with *Geotrichum candidum.* O. R. Irvine, D. H. Bullock, and W. H. Sproule, *Ontario Agricultural College.*

M21 Further studies on the use of ultrasonic waves to accelerate the ripening rate of cheese. J. H. Nelson and W. C. Winder, *University of Wisconsin.*

M22 Effect of temperature and pH on the survival and growth of Swiss-cheese bacteria. R. P. Tittsler, J. Wolk, and R. E. Hargrove, *Washington Utilization Research Branch, U.S.D.A.*

M23 Factors affecting the organic acid production of propionbacteria used in the manufacture of Swiss cheese. I. S. El-Hagarawy, W. L. Slatter, W. J. Harper, and I. A. Gould, *The Ohio State University.*

M24 Free amino and fatty acids in commercial Swiss cheese. P. C. Hintz, W. L. Slatter, and W. J. Harper, *The Ohio State University.*

M25 A low-fat soft-ripened cheese. R. A. Reisfeld and W. J. Harper, *The Ohio State University.*

M26 The heat processing of Italian curd into Mozzarelle cheese. F. V. Kosikowsky and G. J. Silverman, *Cornell University.*

10:30–11:00 A.M.

Combined Sections A and B.
Room 109, Agricultural Building

M27 A study of lyophilization as a means of preserving lactic starters. J. C. Watts and H. E. Calbert, *University of Wisconsin.*

M28 The effect of emulsifier on growth and toxin production of *Clostridium botulinum* experimentally inoculated into surface-ripened cheese. R. O. Wagenaar and G. M. Dack, *University of Chicago.*

11:00 A.M.–NOON

Section Business Meeting.
Room 109, Agricultural Building

1:30–4:30 P.M.

Section A. **Market Milk and Ice Cream.**
W. M. ROBERTS, *Presiding*
Room 109, Agricultural Building

M29 Effect of certain germicides in sanitizing teat cup inflations of milking machines. W. T. Ezzard and F. W. Bennett, *University of Georgia.*

M30 Laboratory study of milkstone formation. Thaddeus Lewandowski, *Pennsylvania Salt Manufacturing Company, Wyndmoor, Pa.*

M31 Susceptibility of milk to copper-induced oxidized flavor and an inhibitory effect of menadione when included in the ration. W. A. Krienke and L. R. Arrington, *University of Florida.*

M32 The effect of certain treatments on the stability of ascorbic acid in milk. J. W. Stull and R. N. Davis, *University of Arizona.*

M33 Effect of ascorbic acid, amber bottles, daylight, homogenization, and pasteurization on the oxidized flavors of milk. E. S. Guthrie, *Cornell University.*

M34 The susceptibility of milk depleted of the total vitamin C content to oxidized flavors, as influenced by the roughages fed to the cow. V. N. Krukovsky, *Cornell University.*

M35 The buffer capacities of skimmilk, buttermilk, and butter serum from the same whole milk source. V. H. Nielsen, E. W. Bird, and Patricia Chandlee, *Iowa State College.*

M36 A method for measuring the efficiency of labor utilization in market milk plants. J. T. Lazar, Jr., and W. M. Roberts, *North Carolina State College.*

M37 A plant study of bulk milk dispenser operation. E. J. Finnegan, E. M. Babb, Jr., and G. C. Wassum, *Virginia Polytechnic Institute.*

M38 The viscosity of milk at selected temperatures as affected by homogenization pressure and type of homogenizer. C. H. Whitnah, W. D. Rutz, and H. C. Fryer, *Kansas State College.*

M39 The identification of fat globules in the internal structure of ice cream. L. F. Cremers and W. S. Arbuckle, *University of Maryland.*

M40 Increasing solids-not-fat in ice cream. A process for the enzymatic hydrolysis of lactose. J. J. Sampey, *Abbott's Dairies, Inc., Philadelphia,* and C. E. Neubeck, *Rohm & Haas Company, Philadelphia.*

1:30–4:30 P.M.

Section B. **Dairy Chemistry.**

G. H. HARTMEN, *Presiding*

Room 119, Osmond Laboratory

M41 A study of the effect of heat upon ionic availability in milk. J. M. Baker, C. W. Gehrke, and H. E. Affsprung, *University of Missouri.*

M42 The use of aureomycin as a bacterial inhibitor for yeast and mold counts. H. C. Olson, *Oklahoma A. & M. College.*

M43 A semi-quantitative spectrographic analysis of the trace elements in whole milk. C. W. Gehrke, J. M. Baker, H. E. Affsprung, and E. E. Pickett, *University of Missouri.*

M44 The xanthine oxidase (Schardinger's enzyme) content of dairy products and its inhibition by heat. G. R. Greenbank, *Washington Utilization Research Branch, U.S.D.A.*

M45 The effect of xanthine oxidase on the properties of dairy products (a preliminary report). G. R. Greenbank, L. V. Rogers, C. F. Hufnagel, and M. D. Middleton, *Washington Utilization Research Branch, U.S.D.A.*

M46 Characterization of milk amylases. E. J. Guy and Robert Jenness, *University of Minnesota.*

M47 The effect of heat on the chemical nature of the material adsorbed on the milk fat globule. Morrison Loewenstein and I. A. Gould, *The Ohio State University.*

M48 The lipolytic enzymes of raw skimmilk. D. P. Schwartz, I. A. Gould, and W. J. Harper, *The Ohio State University.*

M49 Homogenization and deaeration of milk by ultrasonic waves. Marie Burger and W. C. Winder, *University of Wisconsin.*

M50 Decarboxylation products of amino acids in Cheddar cheese. G. J. Silverman and F. V. Kosikowsky, *Cornell University.*

M51 Application of ion exchange chromatography to the measurement of free amino compounds in various lactic acid starter cultures. M. K. Hamdy, W. J. Harper, H. H. Weiser, and G. L. Stahly, *The Ohio State University.*

4:30 P.M.

Section Business Meeting.
Room 109, Agricultural Building

Thursday, June 24

8:30–10:30 A.M.

Section A. **Milk Fats.**
A. J. MORRIS, *Presiding*
Room 119, Osmond Laboratory

M52 Fatty acid chain-length specificity of some microbial lipases. J. C. Wilcox, W. O. Nelson, and W. A. Wood, *University of Illinois.*

M53 Evaluation of the acid degree of fat and extraction-titration methods for the estimation of free fatty acids in rancid milk and cream. N. P. Tarassuk and E. N. Frankel, *University of California.*

M54 Reichert-Meissl numbers of butterfat produced in Kansas. W. D. Rutz, W. H. Martin, and G. H. Whitnah, *Kansas State College.*

M55 Lactones and the coconut-like off-flavor of milk fat. P. G. Keeney, C. T. Herald, and S. Patton, *The Pennsylvania State University.*

M56 Detection of milk fat adulteration. Mark Keeney, *University of Maryland.*

M57 Reliability of the Reichert-Meissl and Polenske methods for detecting substitute fats in ice cream. G. M. Kloser and J. J. Sheuring, *University of Georgia.*

M58 Compounds in oxidized milk fat producing the kreis and thiobarbituric acid reactions. Mark Keeney, *University of Maryland.*

8:30–10:30 A.M.

Section B. **Concentrated Milks.**
G. H. HARTMAN, *Presiding*
Room 109, Agricultural Building

M59 Effect of heat treatments on antibiotic content of milk. K. M. Shahani, I. A. Gould, H. H. Weiser, and W. L. Slatter, *The Ohio State University.*

M60 The flavor of fresh concentrated milk. E. S. Guthrie, *Cornell University.*

M61 The use of a Venturi atomizer in spray drier design. V. H. Townley and S. T. Coulter, *University of Minnesota.*

M62 Oxygen uptake by milk and milk constituents. A. Thomas, S. T. Coulter, and R. Jenness, *University of Minnesota.*

M63 Use of sucrose in the preservation of frozen condensed skimmilk. T. J. Mucha and R. W. Bell, *Washington Utilization Research Branch, U.S.D.A.*

M64 A mechanical low energy method for determining ease of dispersion of milk powder. W. K. Stone, F. S. Hirt, K. R. Wood, and J. M. McIntire, *Food and Container Institute for the Armed Forces, Chicago.*

M65 Dispersibility of spray dried milk powder as affected by manufacturing conditions. U. S. Ashworth, J. M. McIntire, Glen Watson, Hans Gunthardt, and H. A. Bendizen, *Washington State College.*

M66 Comparison of baking quality of frozen condensed and dried skimmilk. L. V. Rogers and T. J. Mucha, *Washington Utilization Research Branch, U.S.D.A.*

10:30 A.M.–NOON

General Association Business Meeting.
Room 121, Sparks Building

1:30–4:30 P.M.

G. H. HARTMAN, *Presiding*
Room 119, Osmond Laboratory

Symposium: Dairy Waste.
Chairman: C. A. IVERSON, *Iowa State College*

Theoretical Aspects.
S. R. HOOVER, *Eastern Regional Research Laboratory, U.S.D.A., Philadelphia.*

Measurements of Pollution.
NANDOR PORGES, *Eastern Regional Research Laboratory, U.S.D.A., Philadelphia.*
D. G. Reinhold, *The Pennsylvania State University.*

Pilot Plant.
R. H. Jackson, D. V. Josephson, and R. R. Kountz, *The Pennsylvania State University.*

Practical Aspects.
F. J. McKee, *Kraft Foods Company, Chicago*
V. Schwarzkopf, *Lathrop, Paulson Company, Chicago*

Dairy Waste Treatment Plants.
A. J. Steffen, *Wilson & Company, Chicago*
C. W. Watson, Jr., *Dairymen's League Co-op Association, New York*

D. I. C. Waste Disposal Sub-Committee Report.
M. H. Brightman, *Dairy Industry Committee, Washington, D. C.*

PRODUCTION SECTION

Tuesday, June 22

1:30–5:00 P.M.

Section A. **Artificial Insemination and Reproduction.**

P. L. KELLEY, *Chairman*

Room 10, Sparks Building

P1 Fertilization and embryonic mortality rates for bulls with histories of either low or high fertility in artificial breeding. H. J. Bearden, Wm. Hansel, and R. W. Bratton, *Cornell University.*

P2 Field trials with semen containing various combinations of antibacterial agents. E. L. Willett and J. Ohms, *American Foundation for the Study of Genetics, Madison, Wis.*

P3 Some of the influences of mixed ejaculates upon bovine fertility. E. A. Hess, Tom Ludwick, H. C. Rickard, and Fordyce Ely. *The Ohio State University and Ohio Agricultural Experiment Station.*

P4 The use of frozen skimmilk as a semen diluter. Victor Hurst and J. P. LaMaster, *South Carolina Agricultural Experiment Station.*

P5 The longevity of bovine spermatozoa in chemical and heat treated pasteurized milk. E. N. Boyd, J. R. Perkins, Durward Olds, and D. M. Seath. *Kentucky Agricultural Experiment Station.*

P6 The effect of yolk and citrate levels and stage of maturity on the survival of bull sperm at sub-zero storage temperatures. W. C. Kinney, Jr., and N. L. VanDemark, *University of Illinois.*

P7 The effect of cysteine hydrochloride on the livability of bull spermatozoa in unheated skimmilk. P. E. Johnson, R. J. Flipse, and J. O. Almquist, *The Pennsylvania State University.*

P8 Activation of bovine spermatozoa by the use of sodium carbonate. H. C. Rickard, Tom Ludwick, E. A. Hess, and Fordyce Ely, *The Ohio State University and Ohio Agricultural Experiment Station.*

P9 The effect of antibiotics, levels of glycerol and rates of freezing on revival rate of bovine spermatozoa in yolk-citrate and milk extenders. W. E. Erickson, E. F. Graham, and E. C. Frederick, *University of Minnesota.*

P10 The relationship of diluter composition and glycerol equilibration time to survival of bull spermatozoa after freezing to –75° C. Jack Saroff and J. P. Mixner, *New Jersey Agricultural Experiment Station.*

P11 Preliminary fertility results with frozen bovine spermatozoa. R. W. Bratton, R. H. Foote, and Joan C. Cruthers, *Cornell University.*

P12 Effects of levels of sodium citrate, glycerol and equilibrating time on recovery of bovine spermatozoa after storage at –79° C. R. G. Cragle and R. M. Myers, *North Carolina State College.*

P13 Techniques for freezing bull spermatozoa in heated milk and preliminary breeding results. W. T. O'Dell and J. O. Almquist, *The Pennsylvania State University.*

P14 Interference by glycerol with differential staining of bull spermatozoa. J. P. Mixner and Jack Saroff, *New Jersey Agricultural Experiment Station.*

P15 Experimental rations to study the phosphorus requirement of young dairy bulls for normal growth and semen production. P. E. Poitras, S. D. Musgrave, M. Ronning, and R. MacVicar, *Oklahoma A. & M. College.*

P16 Interrelationships among thyroidal activity, sexual behavior, semen production, and the fertility of bulls used in artificial breeding. W. S. Griffith, Cecil Branton, T. E. Patrick, G. F. D'Arensbourg, and H. C. Kellgren, *Louisiana State University.*

1:30–5:00 P.M.

Section B. **Calf Nutrition.**

R. E. Erb, *Chairman*

Room 121, Sparks Building

P17 Studies on the permeability of the gut of the newborn herbivore. H. F. Deutsch and Vearl R. Smith, *University of Wisconsin.*

P18 The value of a combination of antibiotics vs. single antibiotics for young dairy calves. C. A. Lassiter, T. W. Denton, and J. W. Rust, *Kentucky Agricultural Experiment Station.*

P19 Growth and hematological response of calves to antibiotic and arsenical supplementation. F. G. Owen and R. S. Allen, *Iowa Agricultural Experiment Station.*

P20 The effect of different levels of dietary terramycin on the growth of young dairy calves. G. I. Pritchard, W. H. Riddell, and W. B. Durrell, *University of Vermont.*

P21 Effect of aureomycin on certain blood constituents (Hb, packed RBC, Ca, P, RBC, and WBC), body temperatures, weights of organs and tissues, and thickness of small intestine. L. L. Rusoff, F. T. Landagora, and H. H. Hester, *Louisiana Agricultural Experiment Station.*

P22 Results of feeding antioxidants to calves. K. E. Gardner and R. G. Hansen, *University of Illinois.*

P23 Effects of tocopherol administration on dairy calves receiving various filled milks. R. S. Adams, T. W. Gullickson, J. H. Sautter and J. E. Gander, *University of Minnesota.*

P24 Colostrum as a substitute for whole milk in a limited whole milk feeding system. W. S. Gaunya, R. D. Mochrie, H. D. Eaton, and R. E. Johnson, *University of Connecticut.*

P25 The utilization of all the colostrum produced by a dairy herd for feeding the calves. E. A. Keyes, E. J. Peace, and J. L. Brence, *Montana Agricultural Experiment Station.*

P26 Further studies on the use of soybean flour and whey solubles in milk replacement formulas for young dairy calves. J. F. Stein and C. B. Knodt, *The Pennsylvania State University.*

P27 Effect on calves of a whole milk diet supplemented with known nutrients. J. W. Thomas and M. Okamoto, *Dairy Husbandry Research Branch, U.S.D.A.*

P28 Relationship between certain corn products in the diet and the concentration of magnesium in the blood serum of dairy calves. G. E. Hawkins, Jr., *Alabama Polytechnic Institute.*

P29 The effect of management and housing upon growth and health of dairy calves. W. R. Murley, E. W. Culvahouse, and R. K. Waugh, *North Carolina State College.*

P30 A comparison of cottonseed meal, urea, and ammoniated molasses (32%) in the ration of dairy heifers. J. B. Frye, Jr., B. T. Parham, B. L. Kilpatrick, and L. L. Rusoff, *Louisiana Agricultural Experiment Station.*

P31 The value of dicyanodiamide as a nitrogen extender in rations for dairy heifers. N. D. Magruder and C. B. Knodt, *The Pennsylvania State University.*

P32 Further observations on the long-time effects of antibiotic feeding to dairy cattle. N. L. Jacobson, J. T. Miles, and L. D. McGilliard, *Iowa State College.*

Wednesday, June 23

8:30–11:00 A.M.

Section A. **Silage Production and Feeding.**
P. L. KELLY, *Chairman*
Room 10, Sparks Building

P33 The effect on milk production of feeding alfalfa harvested and stored by four different methods. E. A. Keyes and E. P. Smith, *Montana Agricultural Experiment Station.*

P34 The effect on lactating dairy cows of replacing hay with corn silage. R. K. Waugh, W. R. Murley, H. A. Poston, and R. D. Mochrie, *North Carolina State College.*

P35 Lima bean silage for dairy cattle. C. R. Richards, *University of Delaware.*

P36 Composition and nutrient value of grass silage throughout Vermont. J. A. Newlander, *University of Vermont.*

P37 Effect of added ground corn on the composition and palatability of stacked legume-grass silage. R. S. Allen and A. R. Porter, *Iowa State College.*

P38 Ensiling succulent pasture forages with dried citrus pulp. R. B. Becker, J. M. Wing, P. T. Dix Arnold, G. K. Davis, and J. T. McCall, *Florida Agricultural Experiment Station.*

P39 The use of acidifying salts in high moisture hay crop silage. C. H. Gordon, H. G. Wiseman, L. E. Campbell, C. G. Melin, and H. M. Irvin, *Dairy Husbandry Research Branch, U.S.D.A.*

P40 Some chemical characteristics of grass silage made with sodium meta-bisulphite. G. Alderman, R. L. Cowan, J. W. Bratzler, and R. W. Swift, *The Pennsylvania State University.*

P41 Field trials with sodium meta-bisulphite grass silage. J. W. Bratzler, R. L. Cowan, and R. W. Swift, *The Pennsylvania State University.*

11:00 A.M.–NOON

Production Section Business Meeting.
Room 121, Sparks Building

8:30–11:00 A.M.

Section B. **Physiology.**
R. E. ERB, *Chairman*
Room 121, Sparks Building

P42 Using identical twin dairy cows to determine the effect of iodinated casein (Protamone) on milk production, thyroid activity, and body weight changes. E. W. Swanson, R. A. Monroe, and C. L. Comar, *University of Tennessee.*

P43 Results with identical twins in the feeding of thyroprotein. J. W. Thomas and M. Okamoto, *Dairy Husbandry Research Branch, U.S.D.A.*

P44 The effect of feeding different amounts of potassium iodide on the level of protein-bound iodine in cows. J. F. Long, J. W. Hibbs, and L. O. Gilmore, *Ohio Agricultural Experiment Station.*

P45 The effect of restricted roughage, high-concentrate diet upon milk fat, blood glucose, and blood ketones. P. J. Van Soest, N. N. Allen, and L. R. Maki, *University of Wisconsin.*

P46 Studies on the etiology and treatment of ketosis in dairy cows. J. C. Shaw, R. E. Brown, R. A. Gessert, and A. C. Chung, *University of Maryland.*

P47 The influence of intravenously injected calcium borogluconate on the blood levels and urinary excretion of calcium in dairy cows with parturient paresis. J. L. Albright and T. H. Blosser, *State College of Washington.*

P48 Availability of phosphorus from phosphates after irradiation in the pile. L. R. Arrington, J. C. Outler, and G. K. Davis, *Florida Agricultural Experiment Station.*

P49 The modified Richardson biochemical pregnancy test as applied to dairy cattle. Sid Diamond, S. H. Morrison, and J. D. Donker, *University of Georgia.*

P50 Changes in bovine blood proteins associated with parturition. B. L. Larson, G. D. Rolleri, and K. A. Kendall, *University of Illinois.*

P51 The isolation and identification of the causative agent of bovine hyperkeratosis (X-disease) from a processed wheat concentrate. W. Hansel, P. Olafson, and K. McEntee, *Cornell University.*

11:00 A.M.–NOON

Production Section Business Meeting.

Room 121, Sparks Building

1:10–3:20 P.M.

Section A. **Rumen Physiology.**

P. L. KELLY, *Chairman*

Room 10, Sparks Building

The Rumen Story. Film by Ralston Purina Company.

P52 Polysaccharide synthesis and lactic acid production by bovine rumen bacteria. R. N. Doetsch and R. Q. Robinson, *University of Maryland.*

P53 Studies on methane formation by washed suspensions of bovine rumen bacteria. J. J. McNeill and R. E. Brown, *University of Maryland.*

P54 Evidence for an unidentified factor required by a rumen bacterium. R. H. Wasserman, H. W. Seeley, and J. K. Loosli, *Cornell University.*

P55 The effect of washed suspensions of bovine rumen bacteria on the potency of aureomycin and terramycin. P. Jurtshuk, Jr., R. N. Doetsch, and J. C. Shaw, *University of Maryland.*

P56 The effect of aureomycin feeding to dairy cattle on the in vitro digestion of cellulose by the rumen microorganisms. J. R. Lodge, J. T. Miles, and N. L. Jacobson, *Iowa State College.*

P57 The influence of the ration and early rumen development on the volatile fatty acid content of rumen juice and blood sugar levels in high roughage fed calves. H. R. Conrad, J. W. Hibbs, and W. D. Pounden, *Ohio Agricultural Experiment Station.*

P58 Volatile fatty acids in the rumen of cows with ketosis. L. H. Schultz, *Cornell University.*

P59 Yields and composition of holocellulose prepared from various feeds and feces. R. E. Ely and L. A. Moore, *Dairy Husbandry Research Branch, U.S.D.A.*

1:30–3:20 P.M.

Section B. **Joint Session with Extension Section.**

Symposium: Sire Evaluation.

Room 121, Sparks Building

3:30–4:30 P.M.

Joint Committee Reports of Production and Extension Sections.

Room 121, Sparks Building

Thursday, June 24

8:30–10:30 A.M.

Section A. **Pastures and Grazing.**
RALPH E. ERB, *Chairman*
Room 10, Sparks Building

P60 Alfalfa-orchard grass grazing results. R. H. Lush, A. G. Van Horn, W. M. Whitaker, and B. T. Throop, *University of Tennessee* and *Dairy Husbandry Research Branch, U.S.D.A.*

P61 Continuous vs. rotational grazing. A. D. Pratt and R. R. Davis, *Ohio Agricultural Experiment Station.*

P62 How kind of grass pasture influenced persistency of milk production, yield of TDN and intake of dry matter when grazed by dairy cows. D. M. Seath, C. A. Lassiter, Jackson Taylor, and William Templeton, *Kentucky Agricultural Experiment Station.*

P63 Dry matter intake and digestibility studies with grazing dairy animals —a progress report. W. A. Hardison, R. W. Engel, G. C. Graf, and W. N. Linkous, *Virginia Agricultural Experiment Station.*

P64 The grazing behavior of lactating cows on pasture. H. L. Fisher, G. C. Graf, W. A. Hardison, and N. R. Thompson, *Virginia Agricultural Experiment Station.*

P65 Pangola–white clover pastures for dairy cattle. S. P. Marshall, *Florida Agricultural Experiment Station.*

P66 Alfalfa–orchard grass, ladino clover–orchard grass, and ladino clover Kentucky 31 fescue pastures for milk production. N. R. Thompson and C. W. Holdaway, *Virginia Agricultural Experiment Station.*

P67 Biochemical factors that affect the apparent nutritional value of Lespedeza Sericea. G. E. Hawkins, Jr., and K. M. Autrey, *Alabama Polytechnic Institute.*

10:30 A.M.–NOON

General Association Business Meeting.
Room 121, Sparks Building

8:30–10:30 A.M.

Section B. **Genetics.**
P. L. KELLY, *Chairman*
Room 121, Sparks Building

P68 Type score as related to productive life, fat production, and age at first calving. P. L. Kelly, H. P. Davis, and M. N. Lawritson, *University of Nebraska.*

P69 Genetic analysis of components of type and production in Ayrshires. A. E. Freeman and R. S. Dunbar, Jr., *West Virginia University.*

P70 The influence of dams and sires upon the breeding efficiency of their daughters within a Holstein-Friesian herd. M. A. Inchiosa and K. O. Pfau, *New Jersey Agricultural Experiment Station.*

P71 Physiological response and semen production of purebred and F_1 Red Sindhi crossbred Holstein-Friesian and Brown Swiss bulls exposed to extreme climatic conditions. H. Naelapaa, J. E. Johnston, and J. J. Vizinat, *Aluminum Co. of America* and *Louisiana State University.*

P72 Response of Holstein-Friesian, Jersey, Red Sindhi-Holstein (F_1), and Red Sindhi-Jersey (F_1) bulls to a simulated subtropical climate. H. Kellgren, J. E. Johnston, J. B. Frye, Jr., and B. Green, *Louisiana State University.*

P73 Genetic variation and covariation in rate of maturity and level of production in dairy cattle. C. G. Hickman and C. R. Henderson, *Cornell University.*

P74 Genetic covariation between milk yield and fat percentage among herds stratified according to average fat percentage. B. R. Farthing and J. E. Legates, *North Carolina State College.*

P75 A comparison of approximate indexes for butterfat production. R. S. Dunbar, Jr., and C. R. Henderson, *Cornell University.*

10:30 A.M.–NOON

General Association Business Meeting.
Room 121, Sparks Building

1:30–4:30 P.M.

Section A. **Physiology.**
P. L. KELLY, *Chairman*
Room 10, Sparks Building

P76 Histological development in the postnatal bovine testis. R. G. Fossland, *University of Nebraska.*

P77 Beta-hydroxybutyric acid metabolism of the mammary gland. Soma Kumar and S. Lakshmanan, *University of Maryland.*

P78 Recent studies of the antagonism between mammary growth and lactation. Joseph Meites and J. T. Sgouris, *Michigan State College.*

P79 Stimulation of growth and function of the rat mammary gland. W. R. Kirkham and C. W. Turner, *University of Missouri.*

P80 The effect of daily epinephrine injections on lactation in the rat as indicated by growth of the young. N. L. VanDemark and R. L. Hays, *University of Illinois.*

P81 Cause of the differences in the productivity of dairy cattle. C. W. Turner, *University of Missouri.*

P82 Specific activity-time relationship in tracer studies of milk secretion. S. Lakshmanan and Soma Kumar, *University of Maryland.*

P83 ATP-ase activity in lactating bovine mammary particulate. J. E. Gander and W. E. Petersen, *University of Minnesota.*

P84 Oxidative metabolism in lactating bovine mammary particulate. R. S. Adams and J. E. Gander, *University of Minnesota.*

P85 The effect of estrogen and progesterone on the distribution of alkaline phosphatase and glycogen in the bovine uterus. T. R. Wrenn, S. Moss, and J. F. Sykes, *Dairy Husbandry Research Branch, U.S.D.A.*

P86 The agglutination test in the study of the microflora of the bovine vulvovaginal mucosa. J. E. Verna, B. Wasserman, and B. W. Henderson, Jr., *University of Rhode Island.*

1:30–4:30 P.M.

Section B. **Nutrition and Management.**
R. E. Erb, *Chairman*
Room 121, Sparks Building

P87 Undecorticated safflower meal and protein content of grain for milk production. R. H. Ross, G. W. Cleveland, and D. L. Fourt, *University of Idaho.*

P88 Effect of certain factors on the diurnal excretion pattern of chromic oxide. J. C. Mahaffey, W. J. Miller, J. D. Donker, and H. L. Dalton, *University of Georgia.*

P89 An improved method of using plant pigments as an indicator of digestibility. E. A. Kane and W. C. Jacobson, *Dairy Husbandry Research Branch, U.S.D.A.*

P90 A survey of the condition of cows at the time of artificial breeding. F. H. Flerchinger and R. E. Erb, *State College of Washington.*

P91 Calving age and intervals between calvings for successive calvings, first through tenth. H. P. Davis and Benjamin Brost, *University of Nebraska.*

P92 Relationship between number of services and per cent nonreturns, calving rates and sex ratio in artificial breeding. R. H. Foote and Alethea C. Hall, *Cornell University.*

P93 Breeding efficiency as related to number of gestations of cows. M. H. Ehlers, R. A. Morrison, and R. E. Erb, *State College of Washington.*

P94 The effect of the length of intervals between milking on the milk and butterfat production. J. H. Koshi and W. E. Petersen, *University of Minnesota.*

P95 Response of the milking cow to various methods of management immediately following parturition. W. M. Dupre and W. A. King, *South Carolina Agricultural Experiment Station.*

P96 The effect of wind on Holstein, Brown Swiss, Jersey, and Brahman cows. Samuel Brody, *University of Missouri.*

P97 The role of the testis in mammary gland duct growth. R. P. Reece, *New Jersey Agricultural Experiment Station.*

EXTENSION SECTION

Monday, June 21

8:00 P.M.

D.H.I.A. I.B.M. Committee. Open to all. Round table discussion on I.B.M.
Room 10, Sparks Building

Tuesday, June 22

1:30–4:30 P.M.

Opening Business Session, Teaching Methods, and Exhibits.
S. N. GAUNT, *Chairman*
Room 316, Sparks Building

E1 Dairyman's "Bridge of Life." W. A. Dodge, *University of Vermont.*

E2 Use of television in dairy extension programs. Don Murray, *Michigan State College.*

E3 New developments in dairy visuals. G. F. Johnson, *The Pennsylvania State University.*

Presentation and Discussion of Exhibits.
L. H. STINNETT, *Chairman in charge*

Wednesday, June 23

9–11:00 A.M.

Use of Dairy Herd Improvement Associations to Improve Dairying.
E. T. ITSCHNER, *Presiding*
Room 316, Sparks Building

E4 Survey of state D.H.I.A. programs. J. E. Stallard, *University of Wisconsin.*

E5 Survey on weighing and sampling milk for production records when pipeline milkers are used. M. E. Senger, *North Carolina State College.*

E6 New York production and feed analysis. J. D. Burke, *Cornell University.*

E7 Source of nutrients and their effect on production. Charles Chance, *Cornell University.*

E8 Presentation and discussion on changes of D.H.I.A. forms. J. F. Kendrick, *U.S.D.A.*

11:00 A.M.–NOON

Section Business Meeting — Committee Reports.
Room 316, Sparks Building

1:30–3:20 P.M.

Joint Session of Production Section B and Extension Section.

Symposium: Sire Evaluation.

S. N. GAUNT, *Chairman*
Room 121, Sparks Building

Let's look at the record. J. F. Kendrick, *U.S.D.A.*

Limitations of our present methods in selection as influenced by environment. Robert Laben, *University of California.*

New tools as aids in sire selection. J. E. Legates, *North Carolina State College.*

Discussion

3:30–4:30 P.M.

Joint Committee Reports of Production and Extension Sections.

Room 121, Sparks Building

Dairy Cattle Health. R. G. Connelly

Dairy Cattle Breeding. Ralph Corbett

Dairy Cattle Type. F .W. Atkeson

Breeds Relation. R. E. Erb

The Purebred Dairy Cattle Association. R. F. Loree

The National Association of Artificial breeders. H. A. Herman

Thursday, June 24

9:00–10:30 A.M.

4-H Club Work.

E. T. ITSCHNER, *Chairman*
Room 316, Sparks Building

E9 How we encourage 4-H Club members to keep production records. Ralph Bonewitz, *Kansas State College.*

E10 Where do the bull calves fit into the 4-H Club program? Clarence Olson, *North Dakota Agricultural College.*

E11 Some 4-H dairy record keeping results. H. A. Willman, *Cornell University.*

10:30 A.M.–NOON

General Association Business Meeting.

Room 121, Sparks Building

1:30–4:00 P.M.

Dairy Cattle Health and Forage Evaluation.

S. N. GAUNT, *Chairman*

Room 316, Sparks Building

Panel Discussion on Essentials of an Artificial Breeding Disease Control Program. Panel members, Dairy Cattle Health Committee.

Leader, R. G. Connelly

Pasture and Forage Extension Programs.

E12 Suggestions for practical methods of evaluating pastures. George Werner, *University of Wisconsin.*

Open Forum — discussion of pasture, hay and silage evaluation, and merits of zero grazing.

PEOPLE and EVENTS
in the Dairy Science World

Pioneers of the Dairy Industry

One of the great pioneer dairymen of the South is JOHN ALLEN AREY, who retired from active duty as head of dairy extension at the North Carolina State College July 1, 1953. He was born on a farm near Elmwood, N. C., March 12, 1887. For one year he was a student at Lenoir College, and in 1905 he registered at the North Carolina State College. He was graduated with a B.S. degree in 1909 and remained at the College to secure his M.S. degree.

J. A. Arey

Arey's forty years of extension work in service to his native state began in 1913, when he became county agent of his home county. His work there, and more particularly his interest in promoting dairy farming, so attracted the attention of the college authorities that he was called to Raleigh in 1914 to become assistant extension dairyman. He returned to his native county in 1915 to serve as county agent and to help look after affairs on the family dairy farm. In 1920 he returned to the college and was appointed extension dairyman. He remained in this position until his retirement.

During his active years Mr. Arey received many outstanding honors. One of the first acts of the North Carolina Milk Producers Association at its first annual convocation was to present him with a gold watch. The North Carolina State Grange and Epsilon Sigma Phi, national honorary extension fraternity, both awarded him certificates for meritorious service to agriculture. The U. S. Dept. of Agriculture presented him with its Superior Service Award in 1951. Mr. Arey would say, however, that his greatest reward was always the high confidence in which he was held by the dairymen of North Carolina and the friendships he made in 40 years of work with rural families of the state. His word was always accepted as final by the dairymen, and he never knowingly betrayed the confidence of those in any phase of the dairy industry.

He is generally credited with having been largely responsible for the tremendous strides made in dairy farming in North Carolina in the last several years. He also is credited with the development of the dairy herd improvement program, the system of junior dairy cattle shows, the development of artificial breeding, and the promotion of pastures and hay crops to provide cheap roughages for dairy cattle herds.

Mr. Arey is known in almost every farming community of North Carolina. He prepared hundreds of news items and other informational materials for distribution through the usual channels available to the Extension Service. His extension publications were models of practical information, useful to 4-H Club members as well as adult dairymen. His monthly dairy news letter to herd owners in the DHIA and to county agents contained practical advice and workable suggestions.

He himself is a successful dairyman, owning a model farm in his home community. This farm was his only hobby during his years of active work, and his herd of registered Jersey cattle remains his great pride. He still serves as fieldman for the North Carolina Holstein-Friesian Association.

Farm Tanks Popular in Pennsylvania

During the past year farmers have become increasingly interested in farm tanks. At present there are approximately 700 tanks installed, with several hundred more on order for installation.

At the Pennsylvania State University a cooperative project is under way between the departments of Agricultural Engineering and Dairy Husbandry. To date, four different makes of machines have been exhaustively studied for mechanical design and thermal performance, particularly from the standpoint of cooling efficiency. In this study both direct expansion and ice bank types of coolers have been used. In addition, the University Creamery has been actively engaged in a program to have bulk milk tanks placed in use by all farms supplying milk to the Creamery. It is anticipated that this program will be completed within 6 to 8 months.

The University barns are equipped with four tanks which have been in use for the past year. Visitors at the A.D.S.A. meeting in June will have an opportunity to see these working installations, as well as the research program under way.

News from the Chicago Milk Market

According to the March, 1954, issue of the *Reporter* (published by the Chicago Market Milk Administrator) the producer milk receipts for February at 41 pool plants were 13.5% higher than for the same period 1 year ago. The number of producers shipping to the market was 438 greater than for February, 1953; the average daily delivery per producer was 50.9 lb. higher. Prices of standard grade milk delivered to homes in 25 major cities early in March averaged $0.228 per quart, which is $0.004 below the March, 1953, average.

The *Reporter* gives the following data on vegetable fat utilization in the Chicago market.

Utilization of vegetable fat, butterfat, and total fat in frozen desserts by order 41 handlers, May, 1952, through September, 1953

Year and month	Vegetable fat	Butterfat	Vegetable fat and Butterfat	Vegetable fat as a per cent of total fat
1952	*(lb.)*	*(lb.)*	*(lb.)*	*(%)*
May	27,646	811,558	839,204	3.3
June	77,029	1,018,778	1,095,807	7.0
July	116,547	1,059,149	1,175,696	9.9
Aug.	99,599	786,650	886,249	11.2
Sept.	87,296	754,529	841,825	10.4
Oct.	63,895	584,299	648,194	9.9
Nov.	49,548	512,016	561,564	8.8
Dec.	56,042	617,821	673,863	8.3
1953				
Jan.	72,818	521,247	594,065	12.3
Feb.	71,281	512,855	584,136	12.2
Mar.	91,338	630,532	712,870	12.7
Apr.	92,245	633,264	725,509	12.7
May	102,922	742,856	845,778	12.2
June	151,351	988,216	1,139,567	13.3
July	147,149	997,699	1,144,848	12.9
Aug.	150,002	856,737	1,006,739	14.9
Sept.	122,157	788,095	910,252	13.4

Board Assigned to Peru Advisory Work

L. M. BOARD, chief of the Division of Sanitation, Department of Health, Education, and Welfare, has been assigned to duty in Lima, Peru, as regional sanitary engineer advisor, for the Institute of Inter-American Affairs of the Foreign Operations Administration. Mr. Board will render advisory service in sanitary-engineering matters to the health missions in 18 South and Central American countries.

Acting chief of the Division is M. C. HOPE, who has been assistant chief since May, 1953. The Division of Sanitation is responsible for interstate-carrier sanitation under the interstate quarantine regulations and conducts field studies and investigations in milk and food production and distribution, refuse collection and disposal, home-accident prevention, and other problems in the field of environmental health.

Kentucky Adopts A.D.A. Legislation

At the recent session of the Kentucky Legislature, an enabling law was adopted which legalizes the collection of funds on a year-around basis for the support of advertising, promotion, and research for dairy products. In order for the program to become effective, a majority of the producers of dairy products in Kentucky must vote in favor of it. The new law provides that a Dairy Commission be established which shall consist of 4 dairy farmers, the Commissioner of Agriculture, and the head of the Dairy Section of the Univ. of Kentucky. The Commission will conduct the referendum of the producers and, if the vote is favorable, then proceed to collect money to support the program already launched by the American Dairy Assoc.

The check-off from producers is to be set by the Dairy Commission but cannot exceed 2¢ per 100 lb. of milk, or ½¢ per pound of butterfat. This legislation was enacted at the insistence of dairy leaders after a successful 1953 A.D.A. program, in which a voluntary June and July set-aside exceeded $90,000.

Kentucky Dairy Products Association Meets

The 29th Annual Meeting and Industrial Conference of the Dairy Products Assoc. of Kentucky was held at the Brown Hotel, Louisville, on March 10 and 11, with president C. H. CARPENTER presiding. The meeting was attended, not only by Kentuckians but also by

members from the adjoining states of Indiana, Ohio, and Tennessee. Principal speaker on the first day's program was J. O. CHRISTIANSON, Univ. of Minnesota.

In the evening the group met with the Tri-Cities Dairy Technology Society for the latter's monthly dinner meeting and program. MIKE HALES, of Chr. Hansen's Laboratory, discussed "Factors Affecting Development of Cultures and Starters."

On the second day, the ADA Breakfast Festival was one of the highlights, featuring, among other dishes, the ever-popular corn flakes with soft ice cream. The theme of the second day's program was the important problem of creating ways and means for stimulating greater consumption of fluid milk.

Oregon Events

Seven dairy students from Oregon State College took a 2,700-mile, 9-day trip into southern California during the spring vacation. They were accompanied by ROY STEIN, assoc. professor of dairy husbandry and J. O. YOUNG, research assistant.

The group were guests of the Carnation Co. for 3 days in Los Angeles and for 2 days in the Oakland–San Francisco area. Dairy plants, dairy farms, and allied industries were visited.

The Oregon Dairy Breeders Assoc. has recently published their 1954 edition of *Cattle-log*, listing pictures and pedigrees of bulls now in service.

The Oregon Dairy Council reports that 10,000 Dairy Council dental leaflets were used in Portland schools during dental health week.

Plans are being formulated for the 35th annual meeting of the Western Division of the A.D.S.A. to be held in Corvallis July 11-14, 1954.

Benson Addresses Utah Dairymen

Secretary EZRA TAFT BENSON addressed a huge audience of Cache (Utah) Valley dairymen at Logan, April 3. The event was in the nature of a homecoming for the Secretary and some members of his family.

According to Mr. Benson, if we had consumed as much fluid milk and cream per capita in 1953 as we did in 1945 we would have used 7.5 billion more pounds of milk. He further pointed out that if all the dietary needs of our people were being met there would not be enough milk to go around. The remedy of our surplus problem lies in doing a better job of selling milk and its products, Benson stated.

Through proper breeding, feeding, sanitation, and culling of cows, the Cache Valley Dairy Assoc. and Breeding Assoc. have brought about a per cow production nearly double that of the average for the nation. It is this type of progress, stated Benson, that will enable agriculture to share more abundantly in a broad program for a stronger America. The Secretary closed his speech by pledging to every farmer in America that he would never knowingly advocate or support any program or policy not in the best interests of farmers.

According to the 1950 Census

Milk cows were kept on 3.6 million farms
1,704 thousand farms had 1 or 2 cows
1,321 thousand farms had 3 to 9 cows
473 thousand farms had 10 to 19 cows
183 thousand farms had 20 or more cows
17 thousand farms had 50 or more cows

Cottage Cheese and Buttermilk Conference and Clinic Held at V.P.I.

The second annual Cottage Cheese and Buttermilk Conference and Clinic was held at the Virginia Polytechnic Institute March 17-18. Twenty students attended the 2-day meeting which consisted of lectures on "Overnight set method of cottage cheese production," "What cottage cheese and buttermilk mean to the consumer," "Bacteriological fundamentals of lactic culture preparation," "Cultures and bulk starter milk," and "Compositional requirements of cultured products."

Clinic sessions were devoted to the evaluation of cottage cheese and buttermilk samples submitted by companies sending representatives to the meeting.

Featured speaker for the conference was R. L. ZIEGLER, Topeka, Kansas. Other speakers were M. W. JEFFERSON, State Dairy and Food Division, and the following members of the V.P.I. staff: G. C. GRAF, head of the Dept. of Dairy Husbandry; L. B. DIETRICK, dean of agriculture; E. J. FINNEGAN, assoc. professor of dairy manufacturing; and G. J. NAGEOTTE, assoc. extension dairy products specialist.

Crest Foods Co. Announces Officers

Crest Foods Co., Ashton, Ill., manufacturers of special products for use in the food industry, has announced the following company officers: T. D. GASKINS, president; E. C. SCOTT, vice-president and sales manager (stabilizer division); G. W. MCDONALD, vice-president and secretary; JOHN MEINERS, salesman (protein division); and B. R. WEINSTEIN, research manager.

Virginia Dairy Fieldmen Meet

The Dairy Fieldmen's Assoc. of Virginia held its 10th annual conference at V.P.I., Blacksburg, March 25-26, 1954. The association ex-

perienced the largest attendance (75) in its 10-year history. The annual conference of the association is sponsored by the Virginia Dairy Extension Service. The theme of this year's meeting was "Quality and Economy in Milk Production."

Topics discussed during the 2-day conference were: "Quality milk production," G. J. NAGEOTTE, associate extension dairyman, V.P.I.; "The dairy industry and public relations," P. M. REAVES, professor of dairy production, V.P.I.; "Efficient milk production," W. D. HUNNICUT, National Dairy Products Co., New York; "Progress under new manufacturing milk regulations in Virginia," C. F. HANGER, Dairy and Foods Division, Virginia Dept. of Agriculture; "New ideas on low-cost dairy buildings for manufacturing milk," H. L. HUNT, district fieldman, Pet Milk Co.; "V.P.I. dairy research," W. A. HARDISON, associate professor of dairy production; "Low-cost grade A farm structures," G. D. KITE, associate agricultural engineer; "Observations on dispenser handling of milk at V.P.I.," E. J. FINNEGAN, associate professor of dairy manufacturing; "Pasture and forage management," W. W. LEWIS, extension agronomist; and "The dairy fieldman's interest in artificial breeding," W. H. ARMSTRONG, Va. Artificial Breeding Assoc.

New Process Promises Greater Uniformity in Cleaners

Development of an entirely new process of manufacture for sanitation chemicals has just been announced by the Diversey Corp. of Chicago. This new method, called "DIO-MIX," blends ingredients to obtain maximum uniformity.

Instead of various sized, mechanically-mixed powders, flakes and granules, Dio-Mixed cleaners are identical in particle size. Thus, the problem of large, hard-to-dissolve particles at the top of the drum grading down to fine powders at the bottom has been eliminated.

In addition to uniformity, Dio-Mixed cleaners are claimed to be nondusting, free-flowing, free-rinsing, and completely water soluble with excellent grease-dissolving and water-softening properties.

Minnesota Dairy Products Institute Set for September 15

The Univ. of Minnesota's Dairy Department will conduct its annual Dairy Products Institute, September 15-17, according to J. O. CHRISTIANSON, director of short courses.

W. B. COMBS is the chairman of the event. He says a group of outstanding speakers from all over the country will discuss technological and economic problems facing the dairy industry today.

Morning sessions of the Institute will be spent on problems, and afternoon sessions will be devoted to specific discussions of the various products. Butter and ice cream will be the topics of the afternoon sessions of Wednesday, Sept. 15. Thursday afternoon's discussions will be on market milk, cheese, and condensed and dry milk. Dairy plant fieldmen will meet on Friday, Sept. 17.

Ohio State Holds Dairy Technology Career Day

A dairy technology career day was handled cooperatively by industry groups and the Department of Dairy Technology on April 24. High school seniors from various parts of the state were transported to the campus by industry members. The visitors toured the Dairy Technology building and the campus, had lunch together, and attended a University baseball game.

The Dayton, Ohio, dairy industry group (Dayton Dairy Foundation) and the Maumee Valley Dairy Technology Society have now established dairy technology scholarships for high school graduates. These scholarships supplement those already established in Cleveland, Stark County, and Columbus.

Meetings with high school principals and counselors have now been held in the Akron, Cleveland, Canton, Dayton, Columbus, and Toledo areas. Dinner meetings were sponsored by local dairy groups.

The governing committee of the Ohio Dairy Products Research Fund and the Everett Antrim Fund met at the University on May 11. I. A. GOULD presented the annual report on research in the Department.

A series of staff-graduate student seminars are being held on teaching methods and techniques. Such topics as "Preparing to instruct," "How to instruct," "Chart presentations," "Technical writing," and "Scientific writing evaluations" are being discussed.

Cows on Test in D.H.I.A.

According to the Dairy Herd Improvement Assoc. letter of February, 1954, there were more cows on test January 1, 1954, than at any previous time. At that time there were 2,175 associations in operation with 41,254 herds enrolled and 1,311,698 cows on test.

As compared with Jan. 1, 1953, this represents an increase of 1.1% in number of associations; 0.7% in number of herds; and 6.5% in number of cows on test.

California, with 193,500 cows on test, which is 21.5% of the total in the state, leads in number of cows being tested. Pennsylvania, with 4,603 herds on standard test, leads in number of herds being tested.

News from North Carolina State College

North Carolina State College has been fortunate in having some of the top dairy scientists spend their sabbatical leave studying on the campus. The opportunity for the exchange of ideas and thoughts on various topics is stimulating to both the resident staff and the visiting professors.

S. E. SMITH, nutritionist in the Animal Husbandry Dept. at Cornell Univ., spent 6 months on the campus studying the bloat problem in livestock. His complete summary of the literature and suggestions on attacking the problem will be of value to those who devote future research to the subject.

H. D. EATON, nutritionist in the Dairy Husbandry Section at the Univ. of Connecticut, came to the campus in February, 1954, and plans to spend a year studying in the Experimental Statistics Dept. V. A. RICE, head, Animal Husbandry Dept., Univ. of Massachusetts, arrived in February, 1954, and is also studying in the Experimental Statistics Dept.

Another outstanding visitor is W. L. BOYD, dean emeritus, School of Veterinary Medicine, Univ. of Minnesota and a past president of the American Veterinary Medical Assoc., who has spent several months on the campus in an advisory capacity in establishing the Disease Diagnostic Laboratory.

Complacency

A Guest Editorial

The dairy industry has been too complacent. It has taken for granted that consumers must and will buy dairy products for health reasons regardless of quality and methods used in merchandising. Other successful food industries have been developed by aggressiveness and modern production and merchandising methods, and with considerable emphasis on quality. Consumer satisfaction is the basis on which many essential industries have achieved their importance in our American economy. Why should the dairy industry be complacent and deviate from this proven path to success?

J. A. Nelson

The over-all lag of about 5% in dairy products consumption under that of production has resulted in rather widespread pessimism in the dairy industry. For some reason, the milk consumption rate dropped during the period 1940-1952. It required 109.5 billion lb. of milk to satisfy the 132 million consumers in 1940 and only about 5% more, or 115.1 billion lb. to satisfy the consumer demand in 1952. The population increased over 20% during that period, but the percentage increase in total milk consumption was much lower. Naturally, a surplus of dairy products was the result of this decrease in the rate of total milk consumption.

The decrease in the per capita consumption of butter from about 17 lb. to less than 9 lb. during the period 1940-1953 is blamed for much of the dairy products surplus. Economists state that the butter surplus is the result and not the cause of the present dairy products surplus problem. They declare that it is natural to have a surplus of butter when the per capita consumption of milk does not keep pace with the increase in population. Milk fat naturally finds its way into butter when a more profitable market cannot be found for it.

Again, there is pessimism in the industry in regard to "filled" or "imitation" dairy products due to cheaper vegetable or animal fats displacing milk fat in ice cream, cheese, and evaporated milk. Some states have legalized filled or imitation ice cream. This means that these products can be sold and shipped within the borders of those states. There is also fear that companies that process vegetable or animal fats will sponsor laws which will make interstate shipments of imitation dairy products legal and thus make them a threat in a greater number of dairy product markets.

Since the per capita consumption of milk has not kept pace with the rate of population increase, is it not apparent that the dairy industry has done an inadequate job of selling consumers on the health-giving qualities, versatility, economy, and goodness of dairy products? The publicity on the merits of dairy products has been made somewhat less effective by the low quality of some of the dairy products going to the markets. The detrimental effects of poor quality butter, cheese, ice cream, market milk, and other dairy products must be entirely overcome before the industry can realize its full and just share of the food market.

The dairy industry has always believed in quality but not to the extent that it was willing to sacrifice to make it a reality. In spite of the passing of numerous milk and cream grading laws in some states, slow progress has been made in giving quality top priority. Until dairy products are sold on the basis of quality, advertising and merchandising will not be fully effective. Consumers will pay the price for commodities they like and from which they derive satisfaction. Since price is the primary appeal in the sale of imitation dairy products, quality and appealing flavor should be made the dairy industry's biggest sales asset in gaining new consumers, holding old consumers, and increasing per capita consumption of dairy products. Along with quality, consumers should be constantly reminded of the economy, food

value, and health-promoting qualities of these products. They also should be reminded that dairy products comprise 30% of the food consumed but require only 15% of the financial expenditure for food.

By the use of new machinery and methods, and by streamlining production and distribution, the dairy industry can continue to improve efficiency and thereby keep pace with other food industries. In this manner the industry will be in a position to offer the consumer a better and more economical food buy.

An expanded research program directed toward new uses for milk, improved processing, marketing, merchandising, and protection of our present markets is a real need in the dairy industry. Producers and processors alike should cease thinking about milk in terms of butterfat and start thinking in terms of milk fat. Milk should be sold on the basis of total food solids in order to give full value of its nutritional and health-giving qualities. Likewise, the production records of dairy cows should be reported on a total food solids basis in order to give the dairy cow due credit for the amount of food she produces.

It is very evident that the dairy industry has a big job ahead. New dairy products designed to use more whole milk and more effective ways of promoting and merchandising dairy products must be found. Quality must be given first priority. With what the industry has to offer in the way of top value foods and with more consumers being born every day, there is no need for pessimism. All industries have problems and they usually find ways to solve them. We must have faith in our industry, but the Bible says "Faith without works is dead."

J. A. NELSON, *head*
Department of Dairy Industry
Montana State College

LETTERS TO THE EDITOR

New Zealand Dairy Group to Celebrate Silver Jubilee

The members of the New Zealand Dairy Science Association have requested that I write to you with the idea of promoting contacts with the members of your Association. To that effect, a copy of the Proceedings of our last Annual Conference has been sent to you by surface mail.

You might also be interested to know that our Association will be celebrating its Silver Jubilee during the next Conference to be held from the 10th to the 12th of August, 1954, at Massey Agricultural College. Should any of your members be in New Zealand during August, a very cordial invitation is, of course, extended to them to attend the Conference.

Wishing your Association much continued success in the future.

With kind regards,

JACK C. DACRE.
Hon. Sec.-Treas.

Name of Journal Incorrectly Stated

We have received from Dr. G. H. Wilster a copy of a letter to the editor which he wrote and which was published in the February, 1954, *Journal of Dairy Science*. Dr. Wilster quoted our name as PACIFIC FOODS REVIEW. Our correct name is WESTERN DAIRY FOODS REVIEW.

If possible, we would appreciate it, if you could carry a correction in connection with this letter, calling attention to our true name.

Thank you for your attention.

Very truly yours,

VIRGINIA JONES BAKER
Editorial Department

Need for a Rapid Protein Test

Is a new solids-not-fat test what we really need at this time? Why attempt to develop a "Babcock Test" for solids-not-fat when a rapid protein test would probably be more valuable? Many dairymen are already interested in breeding for high nonfat milk solids. Doesn't the present emphasis on a new solids-not-fat test infer that a pound of lactose is equal in nutritive value to a pound of milk protein? Although human milk is higher in lactose than cow's milk, cow's milk is rarely fed to infants nowadays without suitable modification. In making cheese, most of the lactose is lost in the whey. In condensed milk a low lactose content is desirable. High lactose is a problem in the manufacture of ice cream. Even large amounts of lactose in candies tend to crystallize as the candy ages. And, certainly, lactose is not a cheap source of by-products like lactic acid. It is true that lactose is a valuable food that adds just the right amount of sweetness to milk, but most nutrition textbooks stress protein as the most indispensable of the big three ingredients in milk.

Although the amount of lactose secreted by the cow seldom varies much as a result of her environment, except in cases of mastitis, considerable variation in the lactose content of milk between cows has been reported. As we realign our sights for less butterfat and more solids-not-fat in milk, shouldn't we also be thinking about the ratio of protein to lactose in milk? Do we want to breed for the 1.5:7 ratio of protein to lactose in human milk, or the 4:1 ratio in reindeer milk, or should we attempt to maintain our present ratio? If, from now on, protein is to be considered the most valuable major ingredient in milk, why

not try to devise a quick test for protein, rather than solids-not-fat which we may wish to discard in a few years?

DWIGHT ESPE
Office of Experiment Stations

What Is Quality in Dairy Products?

Not long ago the president of a Quality Milk Association stated; "I wish I could say that quality in milk and ice cream is the most important thing wanted by the consumer, but I can't. The number one consumer demand is taste."

When are the people in the dairy industry going to realize that to the consumer, quality is taste? For years we have considered quality as low bacterial content, safety, good cream line, and in some products, body and texture. A change in this thinking is long overdue. Freedom from pathogenic organisms is, of course, a must. However, who cares about low bacterial count if milk is oxidized? What consumer worries about cream line if the milk is rancid or has a strong feed flavor? How many people have turned to margarine because it is difficult to procure butter that has an appealing taste? Who likes smooth, slick ice cream if it does not taste good? With regard to ice cream, what has contributed more to the tremendous expansion of this branch of the dairy industry than its wonderful flavor?

What has been done about improving the flavor of milk? Very little. Certainly a great deal of research has been done on the subject but very little of the knowledge gained has been put into actual practice. The greatest number of consumer complaints today are about the taste of the product. Is anyone in the industry doing anything about this? In a recent survey, it was noted that of 11,000 cans of milk received, only one was rejected. This survey covered ten milk dealers, and 79% of the milk possessed off-flavors. At a judging contest, it was necessary to go through 84 qt. of milk to find one that would score perfect on flavor. In a visit to a certain section of the country, I was amazed to find a great deal of the milk and some of the ice cream with a wild onion flavor. Why weren't the weeds responsible eradicated from the pastures? More difficult problems have been solved. In another survey, 44% of a large milk supply had feed flavor caused by the cows eating silage before or during milking. Some of this milk was so bad that consumers were returning it. The milk met all the local regulatory standards as to butterfat, bacterial content, and coliform. Nothing was being done about the flavor.

The consumer is not going to take this. In the competition for a share of the consumer's dollar, dairy products will have to taste good. They must not only be free from objectionable flavors but the flavor must be better than mediocre. The consumer is being continually reminded about the food value of dairy products. If they are to consume more of these fine foods, it will have to be because they like the taste. If, for instance, people are to choose milk as a beverage, it must *taste* good. It has been stated that if every person in this country were to drink an extra glass of milk a day, the present surplus would soon disappear. This can only be accomplished if all of us make every effort possible to produce and place on the market milk that tastes so good that everyone will want it as a beverage.

ALEC BRADFIELD
Univ. of Vermont

Solvent Extraction Procedure for Determining the Free Fatty Acid Content of Homogenized Milk

Evidence appears to support the recommendation that a suitable solvent extraction procedure be utilized in preference to a churning procedure for obtaining fat from milk or cream for free fatty acid measurement. However, it is recognized that a reliable solvent-extraction procedure requires an equivalent recovery of both the fat and the free fatty acids. When applied to normal milk and cream, the method of Johnson and Gould[1] used directly or modified as to sample size and reagent volume[2] appeared to yield satisfactory results. The results have indicated that the solvent extraction procedure yields fat with a higher acid degree than is obtained when the fat was separated by churning.[3] However, in connection with recent studies involving homogenized milk, it was found that unusually high values were obtained. This was thought to be related to a poor recovery of the fat in relation to the free fatty acids. Investigation revealed that an increase in the amount of alcohol used in the test and an increase in extraction time resulted in more quantitative recovery of the fat and more reliable results.

The major modifications of the previously reported method[2] were: *(a)* reduction of the sample size from 60 to 50 ml., *(b)* an increase in the volume of ethyl alcohol from 25 to 65 ml., and *(c)* increasing the time of shaking with the mixed ethers from 30 to 60 seconds. As

[1] JOHNSON, B. C., AND GOULD, I. A. Milk Lipase System. III. Further Studies of the Solvent Extraction Procedure for Obtaining Fat from Milk for Titration. *J. Dairy Sci.*, 32: 447. 1949.

[2] THOMAS, W. R., HARPER, W. J., AND GOULD, I. A. Free Fatty Acid Content of Fresh Milk as Related to the Portion of Milk and Stage of Lactation. *J. Dairy Sci.* In Press. 1954.

[3] JOHNSON, B. C., AND GOULD, I. A. Milk Lipase. III. Comparison of Solvent Extraction and Churning Methods for Obtaining Fat from Milk for Free Fatty Acid Measurement. *J. Dairy Sci.*, 32: 435. 1949.

previously reported, 1 g. of fat was dissolved in 10 ml. of alcohol and 20 ml. of Skelly F and titrated with 0.01 *N* sodium ethylate using phenolphthalein for the indicator.

The original solvent extraction method, the Thomas method, and the proposed modified method were compared for ten samples of homogenized milk, and the results for fat recovery and acid degrees for milk are shown in the table.

A comparison of the several solvent procedures for obtaining the fat from homogenized milk for free fatty acid determination

Method	No. of samples	Alcohol/100 ml. milk	Fat recovery av.	Acid degree av.[a]
		(ml.)	*(%)*	
Thomas, *et al.*	10	50	51.1	3.39
Johnson and Gould	10	68	58.1	3.01
Increased alcohol method	10	130	89.8	1.95

[a] Acid degree represents ml. *N* base/100 g. fat.

The results reveal that the recovery of fat increased in direct proportion to the increase in the alcohol used in the extraction. Conversely, the acid degree values decreased as the recovery of fat was increased.

Trials were made to compare the recovery of added free fatty acids from homogenized milk for each of the three methods. In all trials the free acids were added to raw milk prior to homogenization. In one trial, capric acid equivalent to an acid degree of one was added, and in the second trial an equal molar mixture of oleic and palmitic acids was added to increase the titration by one acid degree. The results showed that for capric acid, the recoveries were 90.2% for the proposed method, 82.3% for the Johnson-Gould method, and 76.7% for the method of Thomas *et al.;* for the mixed higher fatty acids, the recoveries were 88, 80, and 73%, respectively. These observations indicate that with lower concentrations of alcohol, the fatty acids are more quantitatively recovered than is the fat. Therefore, in using the solvent extraction procedure for determining the free fatty acids, nearly quantitative recovery of the fat is essential for reliable results.

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OUR INDUSTRY TODAY

Brief Reviews of Current Topics

THE FORTIFICATION OF WHOLE MILK AND SKIMMILK

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A discussion of milk fortification calls for consideration of the interests of both consumers and the dairy industry. The consumer is vitally interested in having an adequate supply of milk at a reasonable cost. He also is interested in any measures proposed for its modification. The dairy industry is primarily concerned with obtaining the largest possible market for its products and therefore is interested in how any proposal for fortification may affect this market. Thus, the nutritional need for fortification becomes a basic question.

It is agreed by most nutrition scientists that certain steps which have been taken in the field of fortification, such as the enrichment of flour and bread, the iodization of salt, and the addition of vitamin D to milk, have proven advantageous for better nutrition and health. At the same time, it is recognized that the unlimited fortification of our food supply with specific nutrients is undesirable. The issues here involved caused the Council on Foods of the American Medical Association in 1939 and the Food and Nutrition Board of the National Research Council in 1941 to adopt certain policies regarding food fortification. In 1953 these bodies jointly reconsidered, in the light of experience and new developments, the policies which they had established over the intervening years. On December 18, 1953, they issued a "Joint Statement of General Policy in Regard to the Addition of Specific Nutrients to Foods," which has since been published.[1] It seems worth while to consider this policy statement in connection with the questions under consideration.

The statement endorses "with carefully defined limitations" the principle of the addition of specific nutrients to certain staple foods for the purpose of maintaining good nutrition, as well as for correcting deficiencies in the diet of the general population or of significant segments of the population. The requirements for endorsement of a specific fortification are stated to be *(a)* clear indication of a probable advantage of an increased consumption of the nutrient in question, *(b)* assurance that the food concerned would be an effective vehicle of distribution to those needing it, and *(c)* evidence that such an addition would not be prejudicial to a good diet in other respects. The statement emphasizes the desirability of meeting nutritional needs by the use of natural foods insofar as practicable. It favors the principle of restoration, that is, additions to the natural levels in foods which have lost nutrients in refining, instead of the addition of higher than natural levels to other foods. It recognizes, however, that there may be cases where the latter is needed. The statement closes with a list of the restorations or fortifications endorsed to date, including the fortification of milk with vitamin D. The report provides the basis on which additional fortifications can be considered for endorsement as the facts seem to merit. This statement has no official standing from the standpoint of controlling the manufacture and marketing of fortified foods. In fact, the Food and Nutrition Board has stated in the case of milk that its decisions should not be interpreted as grounds for prohibiting investigations and market trials with milk modified by the addition of appropriate ingredients in reasonable amounts.

Available Nutrient Supplies Exceed Needs

In considering the nutritional need for the further fortifications of our diet with vitamins and minerals, a basic question arises as to the evidence that important deficiencies now exist. Actually, the nutrient supplies now available to our population are markedly in excess of needs. This fact is brought out by the data in the table for nutrients for which further fortifications are frequently proposed. The supply data are preliminary figures for 1953, which differ little from those of the past three years.[2] The allowance figures are those published by the National Research Council in 1947, calculated in accordance with the age, sex distribution shown by the 1950 census.[3] It should be remembered that these allowance figures represent goals for the adequate nutrition of all and are in excess of average needs.

The data in the table show large surpluses for each nutrient, compared with the allowances. Of course, there is wastage between the

[1] J.A.M.A. 154: 145. 1954.

[2] ..... The National Food Situation, Oct.-Dec., 1953. Bur. Agr. Econ. U.S.D.A.

[3] Data supplied by Dr. Esther Phipard, Bureau of Human Nutrition and Home Economics, U.S.D.A.

TABLE 1

Per capita edible nutrient supply at retail level (1953) compared with per capita recommended dietary allowances

	Vitamin A	Thiamin	Riboflavin	Niacin	Vitamin C	Iron
Supply	7,800	1.87	2.34	19.9	115	17.2
R. D. A.	4,500	1.24	1.62	12.4	70	11.6
Surplus (%)	73	43	44	60	64	40

retail level and actual consumption. Even so, the margin between supplies and allowances is so large as to indicate that on the average our nutrient supplies are more than adequate. A further fact to be taken into account, however, is that many persons consume markedly less than the average, for various reasons, and thus specific deficiencies exist among certain individuals and groups of the population. Both recent dietary surveys and medical evidence bear out this point.

Thus, the nutritional justification for any further fortification of our food supply rests on evidence that it would be effective in correcting the limited cases of deficiency which actually exist, recognizing that no benefit could be expected for the general population. The fact that the cases of deficiency occur primarily among the low income groups raises the question as to whether increasing the cost of a given food by fortification might not actually lessen its consumption. There are also broad questions of nutritional policy involved, such as the desirability of promoting better nutrition through education, which will cause consumers to make better use of the liberal supplies of high quality foods now available.

The discussion thus far has dealt with principles which should be taken into account in considering specific practices and proposals regarding the fortification of dairy products. Various proposals require separate examination.

The Fortification of Whole Milk

The addition of vitamin D to whole milk was endorsed over 20 years ago by the Council on Foods and later by the Food and Nutrition Board on the basis of evidence of the widespread occurrence of rickets in infants and children and the recognition that milk, which naturally contains little vitamin D, would be an excellent carrier for that vitamin. The use of this fortification measure for improving the nutrition of children has been justified.

An entirely different case is represented by the practice in a few markets, which is being considered much more widely, of fortification with a half-dozen or more vitamins and minerals. This multifortification involving formulas comparable in many respects to the complex mixtures now sold in pill form, violates the principles which have been reviewed. It is a shot-gun method of trying to remedy the limited cases of deficiency which actually exist for specific vitamins, without any convincing evidence of any significant benefit. The multifortification of milk can be promoted on an insurance basis, as is now done for complex mixtures sold over the drug store counter. Such a basis does not seem nutritionally sound. In general, it is agreed that there is no benefit from supercharging the body with vitamins beyond any demonstrable needs. The idea of insurance can be extended to a wide variety of recently discovered vitamins, for which there is no evidence of deficiency in the human diet. It is believed that milk should not be made the medium for such developments.

Excess of certain nutrients may lead to malnutrition. Fortification formulas now in use in certain states specify additions of various vitamins and minerals to milk in amounts that provide, in one quart, levels which approximate the full recommended dietary allowances. Yet a quart of milk supplies only a fraction of the day's total food needs. The principle that a single food may be fortified to the extent that it will supply the entire daily need or more of various nutrients can obviously lead to such an excessive fortification of our food supply as to be entirely contrary to policies which should govern diet selection and good nutrition. It is becoming increasingly recognized by nutrition scientists that malnutrition can be caused by nutritional imbalances resulting from excesses of certain nutrients, as well as by actual deficiencies. Excessive and indiscriminate multifortification can have this undesirable result. One can understand the desire of manufacturers of vitamins and various complex mixtures to extend the market for their products. They see in milk a useful tool for the purpose. Thus, it is to be expected that some of these manufacturers are actively promoting milk fortification in the dairy industry on the basis that the market for milk can thus be increased. This is a proposition, especially as it involves multifortification, that the industry should independently examine, particularly as regards long-term effects.

Further glamorizing of milk may obscure its basic value. It is to be expected that, because of the belief of some consumers that the more vitamins they consume the better, and with the support of sales promotion accordingly, there would be some demand in many markets for a multifortified milk. It is argued that milk needs "glamorizing" to increase its sales and

that multifortification would do it. The large number of products now being glamorized on the radio and television causes one to be somewhat skeptical of the long-term benefit of such practices to the milk market. From the standpoint of its most important factor, namely, nutritional qualities, milk needs no glamorizing. It is now rated at the top among foods by nutrition scientists because of both the amounts and kinds of important nutrients supplied. Glamorizing with further additions might tend to obscure milk's basic values. A multifortified milk will probably sell at a premium, and presumably at an increased profit per quart to the dairy. In promoting such a product as being superior to the regular milk, how will the market for the latter be affected? The dairy company and the dairy farmer should carefully consider whether the higher cost product would reach those in actual need of additional vitamins.

The dairy industry is entitled to consider any measure which will supply the consumers with a product more in keeping with their wants, but it should not overlook the fact that nutrition scientists have played a large role in building up the present market for milk. Therefore, the industry should consider carefully how any new promotion measures may reflect on the effectiveness of the arguments already advanced for the excellent food value of milk.

Fortification should be regulated. In connection with any regulation approving fortification, it is important that provision be made for a type of control which will insure that the claimed additions are actually being made. Adequate control measures are needed, both for the protection of the consumer and for the prevention of unfair competition among dairies in cases where the product fails to provide the additions claimed.

Need for vitamin A fortification can be questioned. Apart from multifortification, the addition of vitamin A is being practiced in certain markets. This addition is not subject to many of the questions raised about multifortification, but the practice requires examination on the basis of nutritional need. As shown in the table, the average per capita supply of vitamin A exceeds the recommended dietary allowances by over 70%, a greater surplus than for any other nutrient listed. Nevertheless, deficiencies may occur among some segments of the population, as is indicated by a survey of city diets made by the Bureau of Human Nutrition and Home Economics in 1948-49.[4] The intakes of 14% failed to meet the allowance level. In families with incomes under $1,000 a year the figure was 28%. The data should not be interpreted to mean that actual deficiencies existed to that extent, because the allowance is in excess of average need, and particularly because the ability of the body to store the vitamin means that deficiencies indicated by inadequate intakes over a short survey period may have no significance as regards nutritional status. This ability also largely nullifies the argument that milk is an unreliable source of this vitamin because its content is variable and low in the winter months. Actually, if all the milk consumed had only its minimum winter value, instead of the year-round average of 1,530 I.U. per quart, the average per capita food supply at the retail level still would be markedly in excess of the allowance. Considering these facts, it does not appear that any convincing nutritional case can be made for the fortification of milk with vitamin A for the general public. The nutritional justification for making a fortified product available for correcting deficiencies which may exist among low income groups would appear to rest on the probability that, despite the premium price charged, the product would actually reach those in need.

Addition of vitamin C and iodine inadvisable. Milk is low in vitamin C, and its fortification on this basis has been proposed. The more than 60% surplus in our food supply, compared with the allowance recommended, and the ease with which this vitamin can be obtained from a variety of palatable foods otherwise desirable in the diet suggest that there is no nutritional justification for this fortification. The case is similar for iron, which is also present in milk in relatively low amounts.

The fortification of milk with iodine to meet deficiencies in goiter areas is questionable in terms of public health policy, which recommends iodized salt as both safe and effective for the purpose.

Intrinsic nutritive value of milk should be stressed. In summary, with respect to whole milk it seems that measures which will emphasize the marketing of the natural product to consumers in a wholesome and palatable form, and at as low a price as the operation justifies, will best serve the cause of nutrition and the long-term prosperous industry. Much opportunity remains to capitalize further on the unique, intrinsic nutritive values of milk in the interest of extending its market, particularly for adults. This method will be much more effective in the long run than trying to glamorize it by multifortification.

Such an argument should not be used to deny the dairy industry the freedom, subject to considerations of public health and national nutritional policy, to give the consumer what he wants or to conduct marketing trials to see to what extent fortification might increase the demand. Any such trial should be conducted on a controlled experimental basis and should not be subject to unjustifiable promotion or claims regarding the fortified product, particularly as compared with natural whole milk.

[4] Special Report No. 2, October, 1950.

The Fortification of Liquid Skimmilk

Aside from the fat and fat-soluble vitamins, all the nutrients in milk, at least all that are known to be of nutritional significance, are in the nonfat fraction. Nutrition scientists have recognized that skimmilk provides a means of getting more high quality protein, calcium, riboflavin, and other nutrients into the diet at low cost and thus have advocated its increased consumption, both liquid and dry. It is gratifying to note the increased interest of both the industry and the public to this end.

Addition of nonfat solids has merit. The development, which is now being practiced in some markets and proposed much more widely, of adding to liquid skimmilk more of the nonfat solids in the interest of providing a more palatable product seems a nutritionally sound and desirable one as a means of supplying still more of these nutrients cheaply. In general, the dairy industry should not be denied the opportunity to modify or combine its products in the interest of providing a food which serves nutritional needs and meets a market demand.

Low calorie value of skimmilk should not be overemphasized. The recent recognition of the need for diets low in calories, but adequate in nutrients, for many of our population has stimulated the consumption of skimmilk for this purpose. This use is being advocated for reducing diets by both medical men and dietitians. Certainly, the dairy industry is entitled to capitalize on this market. At the same time, it would seem undesirable to place too much emphasis on the low calorie value of skimmilk as distinguished from its nutrient contributions, which are of primary importance. This can have an adverse effect on the use of whole milk in the diet. Carbohydrates and protein, as well as fat, can produce body fat. In this sense, all foods are fattening. The important consideration is the extent to which a given food supplies the various nutrients. Here, whole milk is outstanding. In supplying approximately 25% of the average per capita calorie allowance, a quart of whole milk also provides 53% of the allowance for protein, 34% of vitamin A, 108% of calcium, 28% of thiamine, 104% of riboflavin, and lesser percentages of several others. There are medical men and dietitians who feel that whole milk should be included liberally in reducing diets as the easiest way to supply the needed nutrients and also to provide satiety value. The promotion of skimmilk primarily as a low calorie food brings it into competition with all kinds of products whose low calorie values are stressed but which are low or nil in important nutrients. For example, there is the advertisement that "A bacardi cocktail has less calories than a glass of skimmilk."

A strong support of fortification not justified. The increasing market for liquid skimmilk has stimulated promotion for its fortification with various vitamins, notably A and D. With respect to D, it is argued that the fortification of whole milk with this vitamin, long recognized as advantageous, should be extended to the nonfat product. Without implying that such a step would be undesirable, it should be pointed out that the cases are not parallel. As previously discussed, the case for the fortification of whole milk with vitamin D has rested primarily on rickets prevention in infants and children. The same case cannot be made for skimmilk because most authorities agree that it should not replace whole milk for children. A promotion of vitamin D skimmilk which might result in such a replacement would seem to be undesirable from the standpoint of both nutrition and the market for whole milk.

Neither is the nutritional argument for the fortification of liquid skimmilk with vitamin D based upon its increasing consumption by adults a strong one. While the essentiality of liberal intakes of the vitamin for calcium and phosphorus utilization during growth is clearly established, this is not true for adults, except for pregnant and lactating women. For others it is commonly stated by nutrition authorities, as a result of their review of the evidence, that the need for vitamin D is minimal. Mention is made of the probable desirability of providing small amounts for those habitually shielded from sunlight. In the table of recommended allowances, no adult allowance is set forth for vitamin D, except for pregnant and lactating women, for whom 400 I.U. is specified. The claim that the fortification of skimmilk with 400 units of vitamin D would serve a nutritionally useful purpose for adults in general remains to be substantiated. It is agreed that if more vitamin D is needed in the diet, it is best added in a milk product which contains the calcium and phosphorus to be assimilated.

Fortified liquid skimmilks now on the market, or proposed, commonly contain vitamin A as well as D. The general question of the need for and usefulness of fortification of our food supply with this vitamin has been discussed in considering the fortification of whole milk. The case for skimmilk is somewhat different. The addition of A could be considered a restoration to replace the amount removed with the fat. The levels in use or proposed involve additions in excess of the average year-round content of the whole product. Claims that skimmilk is made superior to whole milk through fortification would seem contrary to the interests of the dairy industry. For adults for whom a diet containing skimmilk instead of whole is prescribed, and for certain low income groups, skimmilk with vitamin A restored might serve a useful purpose in diet planning, despite the wide opportunity to obtain an adequate supply of the vitamin from other sources. Otherwise, it does not seem that the case for this fortification is essentially different from that for whole milk.

In summary with respect to liquid skimmilk, no strong nutritional case can be made for its fortification with vitamins A and D, nor is there a strong case against it. The almost negligible cost of the ingredients is an argument in favor of the fortification as an insurance measure, but this argument loses force as the premium charged, the aspect in which the dairy is most interested, becomes significant. Dairies should not be prohibited from marketing skimmilk fortified with vitamins A and D if they wish to do so, but strong support for the fortification on nutritional grounds is not justifiable. On the other hand, modification with additional nonfat milk solids can result in real nutritional advantages. The considerations involved in the multifortification of skimmilk, which is now being practiced and more widely proposed, would not seem to be essentially different from those discussed for whole milk.

THE SELF-HELP PROGRAM

MACK GEBERT

Dairy Products Marketing Association, Inc. Chicago

Before attempting to evaluate the "self-help" program of the National Milk Producers Federation, it might be well to review what has gone on before and get a little background history. The self-help idea, of course, is not new. The dairy producers of this country have long attempted to find some way to control milk production and to have a greater share of the responsibility in stabilizing the prices of their products.

Mack Gebert

One of the first attempts goes back to the early '30's, when a group of dairy producers, led by John Brandt, Russell Waltz, and others, worked with the government to find some way to stabilize butter prices. An arrangement was made whereby Land O' Lakes Creameries, with government loans, attempted to stabilize the price of butter by buying at a minimum price all that was offered. This was in 1933. Although their efforts did help to stabilize prices to a degree, the job was obviously too big for one individual company and the project was abandoned.

The next attempt was made by organizing a company called the Dairy Marketing Corporation, which was composed not only of producers but of processors, distributors, and handlers. This attempt failed before it started, as the various factions could not agree on a method for stabilizing prices. The corporation was disbanded, and the producers were convinced that if anything was to be done, it could best be done by a producers' organization alone.

In May, 1938, the producers organized a corporation composed entirely of producer-representatives and incorporated as the Dairy Products Marketing Association. From 1938 through 1942, this association successfully stabilized the price of butter at a fair level for that time. In fact, during these 5 years there was a more nearly stable price for butter than in any other 5-year period of butter-pricing history. The only exception would be, of course, the war years, when there were fixed ceiling prices. Also during this 5-year stabilization period, the per capita consumption of butter was around the highest level — 17 pounds. When World War II came, the stabilization effort for butter was dropped. DPMA was used as a procurement agency under contract with the government for the purchase of butter and cheese needed by the government and our allies.

When war ended, the government decided to carry on the stabilization program itself. This was done, even though up to that time the Dairy Products Marketing Association had successful experience in handling over one billion pounds of dairy products. For a few years after the war, the price of butter remained above support levels and no purchases by the government were necessary. However, prices weakened in the spring of 1949, and for the years 1949 and 1950 the government purchased a total of some 240 million pounds of butter. While piling up these huge stocks of butter, the Department of Agriculture was able to dispose of around 100 million pounds in domestic and foreign relief channels. In addition, during the latter part of 1950, business began to improve and the USDA was able to sell around 140 million pounds of butter through commercial channels to the butter trade. Oddly enough, within a year there was a real shortage of butter again and the retail price advanced to about $1.00 a pound. There was no surplus of butter from that time until November 28, 1952, when the government made its first purchase in this present surplus period. During the years since the government has taken over the stabilization program they have had very little success in actually stabilizing the price of butter. In fact, the period since World War II has been the most unstable in the entire history of butter prices. Ever since the operation of the price support program has been handled by the government, the producers have never given up the idea of getting the government out of the dairy business. They still think they can run a stabilization program more effectively than the government. The current "self-help" program adopted by the National Milk Producers Federation represents the cumulative experience and thinking of these many years.

Details of the Self-Help Plan

This self-help program, in short, would provide a means to stabilize dairy products prices and to handle surpluses on a self-financing basis. It proposes that the dairy farmers, themselves, regulate their own production and provide adequate supplies of milk and other dairy products — and assume any losses which may be incurred in the disposal of seasonal or occasional annual surpluses. Briefly, the program involves:

(1) *The setting-up of a dairy stabilization board of 15 members, to be appointed by the president from nominees selected by the producers.* To be eligible to serve on the board, or to vote for nominees, a milk producer would have to be engaged at least 10 months a year in the selling of milk or butterfat on a commercial basis. The board would be composed of milk producers and officers and full time employees of farmers' cooperative associations. The Secretary of Agriculture would be represented on the board through an ex-officio member.

The Secretary of Agriculture would set up 15 federal dairy districts, giving consideration to complete geographical representation and the importance of dairying in each district. Each district would have one member on the board. Three candidates would be nominated by the milk producers in the district. The president would then select one of the three for appointment to the board. The secretary would conduct all elections for the selection of nominees. In making appointments to the board, consideration would be given to securing an equitable representation of the various forms in which milk and its products are sold.

Board members would serve for 6-year terms, so staggered that five members would be elected every 2 years. Board members would receive a per diem of $50, but not to exceed a total of $2,500 in any one year, plus expenses. The board would meet quarterly and at other times upon call of the chairman. The chairman of the board would be selected by the board and would serve for a term of 2 years. The board could select an executive committee of five from among its members. The board could provide a per diem for members of the executive committee in addition to their compensation as members of the board.

The general powers and liabilities of the board would be similar to those of the Commodity Credit Corporation. The board would be authorized to acquire, handle, and dispose of milk and dairy products. It could conduct its operations through its own staff or corporations, stabilization corporations, brokers, agents, or established trade facilities. It would be directed to give due consideration to disrupting, as little as possible, the usual and customary channels of trade.

The board would be authorized to borrow up to 500 million dollars from the Commodity Credit Corporation. It could also borrow from private credit sources and pledge its assets as security. Aside from the power to borrow from the Commodity Credit Corporation, the board would have no authority to obligate the United States. The board would pay interest on all money borrowed from the corporation at rates equal to the cost of money to the government. The board would submit annual reports of its business to the Secretary of Agriculture and to Congress.

(2) *The establishment of price-support levels at which the board would purchase dairy products that are in surplus in the regular channels of trade.* The level at which prices would be stabilized would be determined annually by the board and announced as far in advance of the beginning of the marketing year as would be practicable. The stabilization level so announced would not thereafter be varied before the close of the marketing year except in emergency cases. In setting price stabilization levels, the board would give consideration to the objectives of the act and to general economic conditions affecting market supply and demand for milk and dairy products. Appropriate differentials could be provided for quality, location, season, and other factors.

(3) *The levying of assessments by the board on all milk or butterfat sold by individual farmers in commercial channels.* The money collected would be used to cover losses on surplus handling, to pay administrative costs, and to participate in research and the promotion of dairy products. The cost of operating the stabilization program would be charged against producers of milk and butterfat for market. Stabilization fees would be determined by the board annually in accordance with the estimated requirements of the program for the ensuing marketing year. Purchasers of milk, butterfat, or dairy products from producers would withhold from the purchase price any amount equal to the stabilization fee and remit the same to the Internal Revenue Service.

Stabilization fees on sales to consumers for other than commercial uses would be paid directly by producers. The Internal Revenue Service would collect the fees and deposit them to the account of the board. Imports of dairy products would be subject to stabilization fees at the same rates as those collected from domestic producers.

The latest available statistics of the federal government would be used by the board in making its findings and determinations. An exemption would be provided from some of the laws regulating government contracts and the operations of government agencies. Members and employees of the board would not be permitted to speculate in dairy products or to divulge confidential information of the board. The stabilization operations of the board would begin April 1, 1955.

Beneficial to Both Consumer and Producer

It would seem that this program would be beneficial to both the consumer and the dairy farmer. For the consumer, it would mean freedom from the taxes involved in government price supports for dairy products, assurance of adequate milk and dairy products, and protection from unduly high prices.

For the dairy farmer, himself, it would mean assurance of year-around stabilized prices without reliance on government price supports; protection from the effects of surpluses resulting from imports or from diversion to dairying from acreage allotment programs, and a friendlier consumer relationship because of denoted willingness to cut free from government dependence.

Actually, the stabilization plan would be along the same lines that DPMA originated and also later used by the USDA since World War II. Prices would be stabilized throuh the operation of a surplus holding pool, purchasing surplus manufactured dairy products and removing them from the ordinary channels of trade. The prices of purchased commodities would be set by the stabilization board and would be high enough to return to producers of milk and butterfat the established stabilization levels for the marketing year.

The surplus products would be held in reserve by the stabilization board until such time as final disposition would be made. Under ordinary circumstances, the surplus products held would never be returned to the normal channels of trade unless they were needed and the price level had advanced above the stabilization level.

If the surplus products are not needed in commercial channels, then the stabilization board would have broad powers to dispose of them in other ways. They could be exported at bargain prices, could be sold or given away to school lunch programs or other relief channels, or in other ways decided upon by the stabilization board. All losses would be borne by the board and charged against the equalization fees collected from the producers of milk and butterfat.

The board would also be authorized to promote and develop new and expanded outlets for dairy products and also appropriate funds for advertising and promotional campaigns and other marketing activities designed to prevent the development of surpluses.

Dairy imports would be in no way restricted by the program. However, American dairy farmers could not be held accountable for surpluses which result from foreign imports. Therefore, during the occurrence of surpluses arising in domestic production, the government would be required to buy from the Dairy Stabilization Board a volume of manufactured dairy products equivalent to any domestic production displaced by imports. In other words, during periods of surpluses, if the government, for various reasons, thinks it advisable to allow imports of dairy products, the dairy farmers would not be expected to pay for their own surpluses and the imports also.

There likewise would be no interference by this program with other government programs for the control of other crops. But, in any marketing year in which dairy surpluses occur, and in which federal acreage allotment programs result in diversion to dairying, the government would acquire for disposal, in other than the domestic markets, a volume of dairy products equivalent to the increased production attributable to the diverted acres. This means, as long as you have production controls on the other crops — with diverted acres going into dairy production — the additional supplies from these diverted acres would not be an expense of the dairy producers.

Summary

This plan would enable more than two million dairy farmers of the nation to pay for their own production stabilization and price control program, through a self-imposed assessment on their own milk or butterfat production. Apparently, there is no power intended for the dairy stabilization board that is not already being used now by the Commodity Credit Corporation in its support operations.

The dairy producers evidently believe that the dairy industry itself can prove a better merchandiser, a better salesman, and a better exporter than the government — which really isn't set up to do a selling job.

This self-help plan represents a move on the part of the greatest segment of agriculture to free the national economy from the burden of agricultural supports. In the light of what has been learned over the last 16 years, it appears that this plan, if enacted into law, may have a good chance to succeed.

A FILTRATION METHOD FOR ROLLER PROCESS DRY BUTTERMILK SOLIDS

D. R. STROBEL AND C. J. BABCOCK

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The increased use of food grade dry buttermilk solids has brought about a need for developing a United States standard for this product. Development of such a standard necessitates filtration to determine the scorched particle content.

In 1951, the authors *(1)* reported a method of filtering roller process nonfat dry milk solids through a lintine disc by using a pepsin-hydrochloric acid solution. This method required holding the sample 20 minutes in a 45° C. water bath and bringing it to a boil prior to filtering. Later *(2)*, a simplified method was reported which makes use of 10% sodium citrate solution as the filtering agent. This method was more rapid and accurate than the pepsin-hydrochloric acid method.

Development of Testing Procedure

Preliminary investigation showed that the sodium citrate method used for filtering roller process nonfat dry milk solids did not produce a filtrable solution of roller process dry buttermilk solids. The pepsin-hydrochloric acid procedure did, but a more rapid method was needed.

These investigations also showed that a satisfactory filtrable solution could not be obtained by using any of the following: X-100 (triton), X-100 + sodium tetra phosphate, calgon (sodium hexametaphosphate), Tween, Tween + sodium tetraphosphate, sodium bicarbonate, ammonia, boric acid, borate, or petroleum ether. These materials were tested by using the agents at different strengths and with various quantities of the samples and where applicable, as hot and cold solutions.

Tetra sodium salt of ethylene diamine tetra acetic acid[1] powder when cold and when in combination with other agents and tested cold failed to produce a filtrable sample solution. A hot solution of this agent, however, was found to produce a filtrable solution. It was possible to filter 25 g. of the sample by the following procedure: The sample was placed in a beaker containing approximately 400 ml. of a 10% solution of the dispersing agent (tetra sodium salt of ethylene diamine tetra acetic acid powder) and heated to 175-180° F.; the hot solution was transferred to a Waring Blendor, agitated for 30 to 45 seconds, and filtered immediately.

[1] A commercial powder known as "Versene" (successfully used in the detergent butterfat test developed by the Bureau of Dairy Industry) manufactured by the Bersworth Chemical Co., Framingham, Mass., was used in these experiments.

The Recommended Method

A shorter and more successful procedure was to place approximately 300 ml. of a hot (175-180° F.) 10% solution of the dispersing agent in a Waring Blendor; turn on the mixer and add 17 g. of sample; add a few drops of diglycol laurate (defoaming agent) and mix 8 to 10 seconds; add hot solution of the dispersing agent to the mixer until the volume is approximately 500 ml. (the maximum amount of solution that can be agitated in the blendor without spillage); agitate the sample for 45 seconds and filter immediately through a 1.25 in. (1.125 in. filtering surface) lintine disc using an aspirator type laboratory tester.

In establishing this procedure, it was found that it is important to use the proportion indicated, as a 5% solution did not successfully filter 17 g. of the sample, and 15 and 20% solutions produced a gelatinous material that clogged the pads and prevented filtration. It was also found that 20 g. of sample failed to filter readily when the shorter method was used.

Summary and Conclusions

A rapid and accurate method of filtering roller process dry buttermilk solids using a hot 10% solution of a tetra sodium salt of ethylene diamine tetra acetic acid powder was developed. This method proved satisfactory with 29 of 33 samples from different plant sources. The four samples that failed to filter contained far in excess of the 32.5 mg. disc of the United States Scorched Particle Standards for Dry Milks.[2] Samples were also successfully filtered by this method at the American Dry Milk Institute Laboratory and at the Dairy Division, Agricultural Marketing Service Laboratory.

[2] U.S.D.A. Scorched Particle Standards for Dry Milks. Sepia photograph, U.S.D.A., 1951.

ACKNOWLEDGMENT

The authors express their appreciation for the assistance of W. G. Bryan and G. H. Norman in conducting some of the tests.

REFERENCES

(1) STROBEL, D. R., AND BABCOCK, C. J. A Filtration Method for Determining Sediment in Roller Process Nonfat Dry Milk Solids. *J. Dairy Sci.*, **34**: 167. 1951.

(2) STROBEL, D. R., BRYAN, W. G., AND BABCOCK, C. J. A Simplified Method for Filtering Roller Process Nonfat Dry Milk Solids. *J. Dairy Sci.*, **34**: 1159. 1951.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASES

490. Milking methods and milk fever. ANON., Miss. Agr. Expt. Sta., State College. 66th Ann. Rpt., 29. 1953.

Cows freshening in the College herd were divided into three groups; Group I, calf remained with dam and cow milked according to normal practice; Group II and III, calf removed from dam before nursing and cow is completely milked with the aid of oxytocin in Group II and without oxytocin in Group III. Only a few cases of udder congestion have been encountered. The different milking practices had no effect on the extent of congestion or the time required for the congestion to disappear.

R. W. Hunt

491. The cells in bovine milk. P. S. BLACKBURN and I. MACADAM, Dept. Pathology, The Western Infirmary, Glasgow. J. Dairy Research, **21**: 31. 1953.

Fore-milk from 5000 samples collected in various stages of lactation from healthy and mastitic cows was examined by the direct-smear technique. The initial stain used was Sudan Black B; counterstaining by the Leishman method. Lymphocytes made up 6 to 20% of the cells found. Red blood cells were found only in blood stained milk. A large epithelial cell was found on occasions. More of the epithelial type of cells were found in milk of late lactation. The cells described were granular leucocytes including probably polymorphs, eosinophils and perhaps basophils, non-granular cells (lymphocytes, non-vacuolated epithelial and vacuolated epithelial cells). Mastitic milk did not appear to differ from normal milk in the cell types found or their differential count.

J. D. Donker

492. The relationship between mastitis and the method of stripping after machine milking. F. K. NEAVE, T. M. HIGGS, D. SIMPSON, J. OLIVER, and F. H. DODD, Bacteriology and Dairy Husbandry Dept., Nat. Inst. Research Dairying, Univ. of Reading. J. Dairy Research, **21**: 10. 1954.

Hand stripping versus machine stripping following machine milking was studied in two commercial herds of British Friesian cows. Each milking was begun with the milking machines placed on animals infected with *S. agalactiae*. The teat cups were not disinfected between cows. The occurrence of mastitis (clinical or determined bacteriologically) in subsequently milked cows was noted and comparisons made between groups. New cases of mastitis were treated with penicillin, leaving only the original source. The number of weeks of exposure per infection was 28.2 and 32.8 for No. 1 herd and 9.5 and 9.6 weeks for No. 2 herd for the hand stripped and machine stripped groups. The new infection rate per cow was 4 times greater in herd No. 1. This higher incidence of mastitis was highly correlated with the presence of erosions near the teat orifice. Clinical cases were recorded more frequently in the machine-stripped group. It was apparent that the great differences in infections between herds was caused by something other than the stripping method employed, i.e. personnel, incidence of sores and abrasions near teat orifices.

J. D. Donker

BOOK REVIEWS

493. Antiseptics, Disinfectants, Fungicides, Chemical and Physical Sterilization. Edited by G. F. REDDISH. Lea and Febiger, Philadelphia, Pa. 841 pp. $15.00. 1954.

This book is a compilation of the work of thirty different authors. The subject material is divided into eight major parts. The first part is devoted to a very brief history of antiseptics and to the problem of definitions. Part two discusses methods of testing phenolic compounds, chlorine compounds, and quaternary ammonium compounds. Methods of assay for each compound are presented and discussed. The result obtained with various test procedures is given in some detail. Practical testing techniques are emphasized. Detailed procedures are given for testing the toxicity of various types of antiseptics and disinfectants. Several special sterility tests are described in addition to those listed by the United States Pharmacopeia. Part three presents rather extensive sections on iodine, mercurials, heavy metals, bis-phenols, essential oils, alcohols, and silver compounds as antiseptics. Major emphasis is given to the surgical applications of these compounds; their use as mildew-proofing agents and in water treatment also is discussed. The

theories concerning the oligodynamic effect of metals are considered in some detail. Various processes utilizing this principle are briefly mentioned and documentary evidence presented to show the efficiency of each process. The use of phenols, chlorine and quaternary ammonium compounds as disinfectants is considered in part four. The discussion on phenols is limited mainly to a theoretical presentation. In the case of chlorine and quaternary ammonium compounds, considerable practical information is given as to their use and effectiveness. Assay procedures for testing chemical disinfectants for surgical instruments are included. A limited presentation of fungicidal and fungistatic compounds is given in part five.

Food and industrial preservatives are considered in part six. Little new information is given on food preservatives. Wool, leather, paint, and paper preservation is described in some detail. Parts seven and eight discuss chemical and physical sterilization techniques. Gaseous sterilization, bacteriological and surgical sterilization by heat, and sterilization by ionizing radiations receive limited treatment. Practical suggestions are included for sterilizing surgical materials. Thermal resistance of microorganisms, food canning and pasteurization are also included.

There is some overlapping of subjects but the data and discussions are not repetitious. The theoretical information on sterilization with steam under pressure is excellent but the detailed procedures for the operation of an autoclave are of limited value in that they apply only to certain models.

The book presents much factual information and is very well documented. It is an excellent source book for teachers, for those interested in testing antiseptics or disinfectants and for those interested in practical problems of disinfection and sterilization, especially in the field of surgery. It is well illustrated with tables and graphs. O. W. Kaufmann

494. Principles of Veterinary Science. 5th Edition. Frederick Brown Hadley, D.V.M. W. B. Sanders Company, Philadelphia, 518 pp., 114 illustrations. 1954.

The text is intended for agricultural students and practical livestockmen and not for professional veterinary students. It is divided into two parts, Anatomy and Physiology of Animals and Animal Diseases.

A chapter giving special attention to meat and milk supplies as they may affect public health officials is included. Within the chapter devoted to Hygiene of Breeding is general information on breeding and related subjects. Part two gives limited information concerning a wide range of subjects of interest to livestockmen, but few of them are presented in sufficient detail to provide him with usable information.

As a text for agricultural students the book is well suited as a source of general information on anatomy and physiology of animals and animal diseases. Few livestockmen would find it a useful handbook of animal diseases affecting his particular class of animals.

The book is printed on excellent paper and is easy to read. The table of contents and index are well done. At the end of each chapter are pertinent questions for review.

E. E. Ormiston

CHEESE

495. Patent developments in the cheese industry — 1900 through 1951. D. M. Irvine and W. V. Price, Dept. of Dairy & Food Industries, Univ. of Wis., Madison. Butter, Cheese, and Milk Prod. J., **44**, 4: 28. 1953.

A complete review of over 600 patents pertaining to developments in the cheese industry is presented in an 80-page bulletin. This bulletin contains abstracts, supported by illustrations of cheese, cheese products, cheese processes and cheese equipment from 1900 through 1951. The patents are classified and cross-indexed by subject matter and patentee.

J. J. Janzen

496. Grass silage and Swiss cheese quality. T. F. Graf, Dept. of Agr. Econ., Univ. of Wis., Madison. Milk Prod. J., **45**, 4: 33. 1954.

Factors affecting Swiss cheese quality are of considerable importance to farmers in Wisconsin. The contention that the feeding of grass silage in itself, is responsible for low quality Swiss cheese, has not been substantiated by data obtained from the major Swiss cheese area of the country — southwestern Wisconsin.

This statement was based on the following findings: (a) The quality of the Swiss cheese manufactured bore no relationship to the amount of grass silage milk received by the factories. (b) When factories with low quality cheese were analyzed individually, it was found that factories using the largest proportions of grass silage milk, had the highest cheese quality. (c) When the quality of cheese made from grass silage was compared to the quality of cheese made from non-grass silage milk, the grass silage cheese was normally higher in quality than was the non-grass silage cheese. (d) It was found that factories using some grass silage milk during the course of the year had a higher cheese quality than those that did not use any grass silage milk at all. (e) The probability that the large difference in cheese quality occurred by chance was extremely remote. Thus, there seems to be a real basis for concluding that the quality of the cheese made from grass silage milk was significantly higher than the quality made from nongrass silage milk. J. J. Janzen

497. Chemical control of cheese mites. R. J. Dicke, K. D. Ihde, and W. V. Price, Univ. of Wis., Madison. J. Econ. Entomol., **46**, 5: 844. 1953.

Several materials were tested for control of two species of cheese mites, *Acarus siro* L. and *Tyrophagus castellanii* Hirst. Spraying and dipping techniques were employed. The only effective material adaptable to the cheese industry was an aqueous solution of the sodium salt of o-phenylphenate. There was little or no effect on the quality of processed and natural cheese.

Wiping or dipping cheese blocks in either mineral oil or cotton seed oil afforded some degree of control. E. H. Fisher

498. Factors influencing grade of cheese. Anon., Miss. Agr. Expt. Sta., State College. 66th Ann. Rpt., 31. 1953.

A study was made to determine whether the fat-to-casein ratio is a major factor in body breakdown and flavor development. Milk was standardized to various fat-casein ratios, starting with 1 part fat to 0.65 parts of casein and continuing to 1 part of fat to 0.9 casein. Results to date indicate that the higher the % of fat to casein, the faster the body breakdown and flavor development. R. W. Hunt

499. What's wrong with the cheese industry? G. P. Wertheimer. Milk Prod. J., **45**, 4: 28. 1954.

The author answers the question of "what's wrong with the cheese industry?" as follows: "There are 2 principal reasons why the average cheese quality is unsatisfactory: (1) the cheeses are not properly cured, (2) the plants I visited employed mostly untrained helpers and only a very few had one well trained cheesemaker."

Solutions suggested involve the correction of reasons (1) and (2). J. J. Janzen

500. Food product flavoring composition and method of preparation thereof. F. H. Stark. U. S. Patent 2,670,293. 17 claims. Feb. 23, 1954. Official Gaz. U. S. Pat. Office, **679**, 4: 991. 1954.

A flavoring material consisting of emulsified fat with an adsorbed layer of colloidal cheese, with the proportion of cheese material limited so that no cheese flavor can be detected.

R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

501. A new market for concentrated milk. Anon. Milk Prod. J., **45**, 4: 24. 1954.

This article discusses the development of a concentrated milk by Iowa State College for the rural market.

The manufacturing procedure is as follows: Grade A milk is pasteurized at 180° F. for 15 sec., homogenized, concentrated 3:1 in a vacuum pan at 125° F., pasteurized and homogenized again and vitamin D concentrate added. For reconstituting, mix 2 parts of water to 1 part of concentrate.

The product is of high quality, has multiple usage and keeps at least 2 weeks in a household refrigerator. The milk consumption in this Iowa community has increased. The retail price of the concentrated milk is the same as 3 parts of regular milk in this area. Deliveries have been on a weekly basis. J. J. Janzen

502. Tailor made dry milk solids. W. F. Donovan, H. P. Hood & Sons, Boston, Mass. Milk Plant Monthly, **43**, 3: 25. 1954.

The nonfat dry milk solids market is very competitive and we must produce what the consumer demands. The demands may be classified as super high heat, high heat bakery, general purpose, consumer-home use, dairy use-cheese, ice cream, etc. The preparation of these various classes and the methods of determining nonfat dry milk solids quality are described.

C. J. Babcock

503. Good buttermilk. C. W. England, C. Y. Stephens Dairy Industries, Washington, D. C. Milk Plant Monthly, **43**, 3: 19. 1954.

In the preparation of good buttermilk, there are 3 steps that must be followed: (1) the preparation of the mother culture, (2) the preparation of the bulk starter, and (3) the preparation of the buttermilk. The 3 rules to observe are: (1) use the best quality high solids milk, (2) use clean, sterile equipment and work in clean, draft-free surroundings, (3) carefully control temperature and time of pasteurization, incubation and cooling, and per cent of inoculum. These steps and rules are fully described. C. J. Babcock

504. Cultured milk powder and method of manufacturing same. E. D. Fear and L. E. Harris (assignors to De-Raef Corp.). U. S. Patent 2,671,729. 5 claims. March 9, 1954. Official Gaz. U. S. Pat. Office, **680**, 2: 488. 1954.

Partially neutralized milk is condensed and then inoculated with a bacillus-type lactic acid-producing organism. After incubating to develop acid to about 1½%, it is dried to form milk powder containing lactic acid.

R. Whitaker

DAIRY BACTERIOLOGY

505. Facultative anaerobic bacteria from the sheep's rumen. S. O. Mann, F. M. Masson, and A. E. Oxford, Rowett Research Inst., Bucksburn, Aberdenshire. J. of Gen. Microbiol., **10**, 1: 142. 1954.

Of 300 facultative anaerobic cultures isolated from the rumen of an hay-fed sheep, 120 streptococci belonged to Lancefield's group D. Of these the majority (82%) resembled *S. bovis* and a few (6%) resembled *S. faecalis*, while the remainder were unclassified by existing schemes. All of the micrococci studied were found to be

coagulase negative. Of the coliform organisms isolated, most of them were found to be typical *E. coli* and several were identified as *A. cloacae*. In addition, several cultures of Gram negative cocci were isolated. J. J. Jezeski

506. The methyl red test in peptone media. M. G. Jennens, Natl. Collection of Type Cultures, London. J. Gen. Microbiol., **10**, 1: 121. 1954.

A number of strains of coliform bacteria were tested for the methyl red reaction on two media. A mineral salts glucose broth produced constant results, whereas a glucose phosphate peptone broth resulted in inconsistent tests with individual cultures. Different brands of peptone did not produce similar results, and media made from the same batch of peptone in successive batches likewise resulted in inconsistent results. J. J. Jezeski

DAIRY CHEMISTRY

507. Enzymic iodination of milk proteins. M. T. McQuillan, R. K. Morton, P. G. Stanley, and V. M. Trikojus. Nature, **173**, 4398: 305. 1954.

Casein and β-lactoglobulin were prepared in pure form from skimmilk and incubated with xanthine, I-131 and KI and either lactoperoxidase or xanthine oxidase. Casein was found to bind 2% of I in the butanol-soluble form, as determined by the radioactivity. Chromatographic fractionation showed more than 90% of the incorporated I was in the form of 3-iodotyrosine. β-lactoglobulin exhibited a very weak I binding capacity in the butanol-soluble form. Experiments indicated the tyrosine residues of β-lactoglobulin are not as favorably situated for conjugation to the iodinated thyronines as are those of casein and that in the iodination of milk, casein is very largely responsible for the I uptake. R. Whitaker

508. Quantitative determination of amino acids using monodimensional paper chromatography. J. F. Roland, Jr., and A. M. Gross, Biochem. Research Dept., The Armour Laboratories, Chicago, Ill. Analyt. Chem., **26**, 3: 502. 1954.

Methods for quantitative amino acid analysis have hitherto required a number of solvent systems for resolution of the acids. A simple method that was used routinely for over a year employed two monodimensional solvent systems to resolve 16 amino acids. The solvents were 2-butanol–3% ammonia (3-1) and 72% phenol. The resolved acids were quantitatively determined by direct photometry of the ninhydrin spots. Determination of the amino acid composition of β-lactoglobulin was found to be in agreement with reported values.

B. H. Webb

509. Reviews of the progress of Dairy Science. Part I. Chemistry and physical chemistry. R. Ashaffenburg and E. R. Ling. **Part II. Rheology and General Physics.** Scott Blair. J. Dairy Research, **21**: 122. 1954.

This comprehensive review covers a total of 611 papers from the period 1950-1953, dealing with many subjects listed under the title headings. Among the discussed phases of dairy science are the composition of milk, butter and cheese, the chemistry of milk constituents (fat, nitrogenous compounds, enzymes and miscellaneous), physical chemistry of proteins, defects in flavor and due to adulteration, analyses of milk and its products (butter, cheese, dried milk, etc.), physical properties of milk, butter and chesee, a consideration of the rheology of the cervical secretions of the bovine and various miscellaneous aspects of different studies related to the dairy field. J. D. Donker

510. Polarographic and amperometric mercurometric determination of disulfide groups in cystine, oxidized glutathione, and proteins. W. Stricks, I. M. Kolthoff, and N. Tanaka, School of Chem., Univ. of Minn., Minneapolis. Analyt. Chem., **26**, 2: 299. 1954.

An accurate and rapid determination of disulfide is of great significance in investigations of biological materials. Procedures are given for the accurate determination of disulfide in cystine, oxidized glutathione and denatured and partially hydrolyzed protein. The method is based on the reaction in alkaline medium in the presence of sulfite of mercuric mercury and disulfide groups. The new method is more rapid, specific and precise than previously used methods. B. H. Webb

511. Amperometric mercurimetric titration of sulfhydryl groups in biologically important substances at the rotated platinum wire electrode and indicator electrode. I. M. Kolthoff, W. Stricks, and L. Morren, School of Chem., Univ. of Minn., Minneapolis. Analyt. Chem., **26**, 2: 366. 1954.

The reaction of mercuric mercury with reduced glutathione and with cysteine has been made the basis of a mercurimetric titration of sulfhydryl in cysteine and glutathione in aqueous medium, using the rotated platinum wire electrode as indicator electrode. The method allows the determination of 7 to 50γ of sulfhydryl in amino acids, peptides, native and denatured proteins with an accuracy of $\pm$ 2%. B. H. Webb

512. Colorimetric method of quantitative microdetermination of quaternary ammonium compounds. Losses of quaternary ammonium compounds caused by glass adsorption and concentration in the foam. J. Fogh, P. O. H. Rasmussen, and K. Skadhauge, Statens Seruminstitut and The Poliomyelitis Inst. of the

Danish Natl. Assoc., Copenhagen, Denmark. Analyt. Chem., **26**, 2: 392. 1954.

The readiness with which quaternary ammonium compounds are adsorbed on a variety of surfaces has caused contradictory results in testing the antibacterial effect of these compounds. A spectrophotometric method of analyzing cetylpyridinium chloride in concentrations ranging from 0 to 25γ per ml. was devised. It was demonstrated that quaternary ammonium compounds tended to be adsorbed onto glass and other surfaces. Treatment of tubes, cuvettes, and pipettes with Plexiglas greatly decreased this adsorption. The method provides a means of determining the very small amounts carried to food products when quaternary ammonium compounds are used for cleaning equipment in the dairy and food industries.

B. H. Webb

513. The physical properties of cow's milk as influenced by stage of lactation. The size distribution of butterfat globules. W. G. Whittlestone, Ruakura Animal Research Sta., Dept. of Agr., New Zealand. J. Dairy Research, **21**: 50. 1954.

Milk was collected from one quarter of a cow throughout lactation to determine the size distribution pattern of the milk fat particles. "Super strippings" were obtained by the use of 30 units of subcutaneously-injected Pitocin following the finish of the normal milking period. Samples were collected periodically throughout the lactation (7 times between 68 and 255 days). Five to ten samples were collected throughout the milking period. There appeared to be no trend in size with progress of milking or in the removal of the "super strippings" occasioned by oxytocin. With the exception that the foremilk, on a few occasions, had fat globules that were smaller than average, there were no trends noted with advancing lactation and no differences noted in the pattern of distribution between P.M. and A.M. milkings.

J. D. Donker

514. Seasonal variations in fatty acid composition of New Zealand butterfat. R. P. Hansen and F. B. Shorland, Fats Research Lab., Dept. of Scientific and Industrial Research, Wellington, New Zealand. Biochem. J., **52**, 2: 207. 1952.

Fluctuations in the fatty acid composition of six butterfats distributed throughout a year were studied by ester fractionation. These samples were taken from the same locality, the analyses made in triplicate and the results subjected to statistical analyses. At the beginning of lactation in July, the C_6-C_{14} acids make up 18.6% and the C_{18} unsaturated acids 29.5% of the total on a molar basis.

By November these proportions had shifted to 26.9 and 23.4 moles % and then near the end of the lactation period gradually returned to near the original proportions. The plane of nutrition and the stage of lactation were the factors which had the greatest influence on the seasonal variation. Statistical evaluation showed significant differences between many of the fatty acid fractions. The greatest differences were between the November and May samples where a significance at the 1% level was noticed in 10 of the 13 differences. There are 6 tables and 31 references. A. O. Call

515. The partition of carotenoids and vitamin A in the milk of cows and goats throughout lactation. (Abs.) R. Chanda, Hannah Dairy Research Inst., Kirkhill, Ayr, Scotland. Biochem. J., **52**, 1: ii. 1952.

The vitamin A and carotenoids were measured in the milk from 3 cows and 2 goats receiving winter rations. In cow milk fat the vitamin A content gradually decreased to the 30th week *post partum*. There was a gradual increase from then to the end of the lactation period. The minimum for the goats was at 20 weeks. The vitamin A in goat milk fat was 98% in the ester form and carotenoids were not present. The variation in the ratio of vitamin A to carotene was almost entirely due to variations in the vitamin A content. β-carotene constituted 65 to 85% of the total carotenoids. Goat milk fat at the second week of lactation contained 60 i.u. vitamin A/g., while in cow milk fat the value for vitamin A plus β-carotene was 49 iu./g. A. O. Call

516. The composition of sow's milk during the course of lactation. D. R. Perrin, Ruakura Animal Research Sta., Dept. Agr., New Zealand. J. Dairy Research, **21**: 55. 1954.

The milk of 44 sows through 68 lactations was analyzed to note trends in the major constituents. The majority of the pigs were of Berkshire breeding. Samples were collected weekly using oxytocin following the secretion of the colostrum. The fat showed a rise in percent for the first three weeks (10.12 to 10.45%) and then a decline to 8.21% over the remainder of the lactation (7-8 weeks). Protein fell from the colostrum period (17%) through the second week (5.31) and thereafter rose to 7%. Lactose declined from 4.85 to 4.31% with advancing lactation. Total solids showed a rise for 3 weeks and then a gradual decline. Ash showed a general rise throughout the lactation period. The fat content showed considerable day to day variation being greater than 2% on several occasions. Samples drawn in sequence following the injection of 3 units of pitocin showed no trends in fat content as is the case with the cow. Solids not fat showed much less variation in samples than the fat content. J. J. Donker

517. The estimation of the energy of sow's milk from fat and total solids content. D. R. Perrin, Ruakura Animal Research Sta., Dept.

Agr., New Zealand. J. Dairy Research, **21**: 63. 1954.

The energy content of sow's milk was calculated from the energy values of chemical constituents comprising the milk. Values from components of cow's milk were used in lieu of figures for sow's milk. Where E = energy (cal/100 g. milk), F = g. fat, L = g. lactose and P = g. protein, E = 9.11 F + 5.86 P + 3.95 L. Since lactose and ash were found to fall within narrow limits with predictable trends, protein may be calcuated by subtracting the predicted lactose and ash and the determined fat from total solids. It was stated that the energy could be calculated by determining directly only the fat and total solids content of milk. Comparisons were made between the two methods of calculating the energy content of sow's milk. Most comparisons were within 1% of each other and the values ranged from 116-155 cal/100 g. milk. J. D. Donker

518. **Testing half-and-half homogenized milks. A suggested procedure.** B. J. Demott, E. S. Humbert, and G. M. Trout, Mich. Agr. Expt. Sta., East Lansing. Mich. State. Quart. Bull., **36**, 3: 291. 1954.

The following modification of the Babcock procedure is recommended for use in testing half-and-half homogenized milks: (1) Use a 9 g., 20% ice cream test bottle; (2) Temper the product and sulfuric acid to 60-70° F; (3) Weigh 9 g. sample directly into bottle; (4) Add 1 ml. normal butyl alcohol and shake; (5) Add 18 ml. of acid in 9, 5, and 4 ml. increments, machine shaking between each inclusion and after the final addition; (6) Centrifuge according to Babcock technique for milk; (7) Temper fat column to 135-140° F; (8) Add glymol and read fat column. R. W. Hunt

519. **Milk fat adulteration.** M. Kenney, Dairy Dept., Univ. of Md., College Park. Ice Cream Trade J., **50**, 2: 26. 1954.

The test involving the crystallization of fat from an alcohol solution followed by a shaking procedure which results in floating fat crystals, in the case of pure milk fat, and no floating crystals in the case of adulterated milk fat, does not function properly if the air temperature is higher than 25° C. An air temperature in the range of 5 to 10° C. also is detrimental to the test because under these conditions adulterated fat yields floating fat crystals. Twenty to 24° C. is the ideal temperature for shaking. W. H. Martin

DAIRY ENGINEERING

520. **Wire mesh conveyor belts.** O. P. Ross, The Cambridge Wire Cloth Co., Cambridge, Md. Ice Cream Rev., **37**, 8: 44. 1954.

Wire mesh conveyors are assisting in speeding production and at the same time meeting the sanitary standards of health authorities in a large eastern ice cream plant.

In this plant wire mesh conveyors are used for bulk production, sandwiches and novelties. Numerous advantages are claimed for the wire mesh conveyor belt.

Although there are nine different weaves of wire mesh conveyor belts, the Baltimore Weave is most widely used in ice cream plants. It is suggested, however, that the ice cream manufacturer should consult with an experienced wire mesh belt engineer in order to select the belt best adopted for the particular job to be performed. W. J. Caulfield

521. **Apparatus and method for filling containers with products.** W. McK. Martin (assignor to James Dole Engineering Co.). U. S. Patent 2,672,270. 14 claims. Mar. 16, 1954. Official Gaz. U. S. Pat. Office, **680**, 3: 712. 1954.

A device for lining up a line of continuously moving flanged cans on a conveyor so the flange of one can overlaps the following can, to facilitate continuous filling. R. Whitaker

522. **Refrigerator apparatus for producing frozen confections.** L. J. Nauroth. U. S. Patent 2,669,852. 4 claims. Feb. 23, 1954. Official Gaz. U. S. Pat. Office, **679**, 4: 864. 1954.

Details are given for the design of a straight line brine tank for quiescently freezing molds containing ice cream, ice milk, and other confections. R. Whitaker

523. **Apparatus for completing and packaging a frozen confection.** F. A. Landgraber, Sr. (assignor to Drumstick, Inc.). U. S. Patent 2,670,887. 7 claims. March 2, 1954. Official Gaz. U. S. Pat. Office, **680**, 1: 183. 1954.

An apparatus for releasing automatically into bags or other containers inverted ice cream cones filled with ice cream or other frozen confection material. R. Whitaker

524. **Closure for containers.** N. E. Spiess, Jr. and L. C. Widdoes (assignors to National Dairy Research Laboratories, Inc.). U. S. Patent 2,670,871. 2 claims. March 2, 1954. Official Gaz. U. S. Pat. Office, **680**, 1: 179. 1954.

An easily inserted container closure for pressure packed products, such as gas whipped cream, is described. The closure may be punctured by a hollow tube which is used to inject propellent gas into the container or to discharge the contents. Upon withdrawal of the tube, the puncture self-seals. R. Whitaker

525. **Mechanism for filling containers with a liquid.** H. H. Franz. U. S. Patent 2,671,591. 4 claims. March 9, 1954. Official Gaz. U. S. Pat. Office, **680**, 2: 446. 1954.

A description is given for the construction of a vacuum type filler suitable for bottling milk and other liquids. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

526. Company credit unions. ANON. Ice Cream Rev., **37**, 8: 144. 1954.

Company credit unions provide a popular and painless way of saving money, in the opinion of several ice cream manufacturers. The credit union is a cooperative venture in which participating individuals invest a part of their wages. Members may then borrow from this fund at a low interest rate or collect interest on their invested funds.

The credit union provides freedom from financial worries to its members and has relieved management of the necessity of advancing funds to employees to tide them over temporary financial emergencies.

Credit union losses resulting from failure of members to repay loans have been extremely small. One company reported a loss of only $20 during eighteen years of operation. Credit unions appear to be most successful where the membership is fairly large. Among nine ice cream firms surveyed, the membership totaled 100 or more per firm. W. J. Caulfield

527. Sound financing for expansion. C. L. FLOUNDERS, Miller Flounders Dairy, Chester, Pa. Milk Dealer, **43**, 6: 52. 1954.

In approaching a sound financing and expansion program, long range planning has many merits. The plan, however, should be reviewed each year and the financing of the fiscal year's program developed. After the expansion program has been developed, the means of financing should be worked out in detail so that the funds will be available as needed. Over-expansion beyond the point that involves a sound financing program, is dangerous and presents difficulties. Details are given for determining the capital needed for expansion and operation.
C. J. Babcock

528. Expectations and plans of milk producers in the Evansville milkshed. W. F. WILLIAMS and N. T. PRITCHARD, Purdue Univ. Agr. Expt. Sta., Lafayette, Ind. Sta. Bull. 594. 1953.

This study was made to determine the nature of the expectations and plans held by a sample of dairy farmers in the Eveansville milk shed. The principal findings and conclusions of the study are as follows: *Uncertainty* (1) Farmers were uncertain of the future and this uncertainty increased as the time span into the future increased. (2) Older producers were less confident and optimistic of the future than younger men. (3) Typical farmer was less uncertain about cow numbers, production per cow and other physical factors, than he was of economic variables. (4) Producers felt that price and technological uncertainties were no greater than those associated with other major farm enterprises.

Price Expectations (5) Price expectations of milk producers were not single-valued. (6) Inaccuracy of price forecast was independent of farm sizes, but older producers were more accurate in their forecasts than the younger men. (7) Price expectations were based on prices of the recent past and projected into the future. (8) Farmers believed that milk prices move up and down with other farm products with the ratio remaining relatively stable. (9) Few farmers, in formulating price expectation, attach probability estimates to price estimates.

Nonprice Expectation (10) Producers were more able and willing to forecast future cow numbers and production rates than to forecast economic variables. (11) Expectations of cow numbers and production per cow were generally too high. (12) Expectations of future cow numbers and production rates were based primarily on present numbers of cows and heifers in herds and on current rates of milk production.

Expectations and Plans (13) All Evansville milk producers take their expectations of future prices into consideration in planning future farm operations, but few attach importance to them, at least in the short run. (14) Reasons for small influence of price expectations on production plans were (a) expectations highly uncertain; (b) farmers believe milk price ratio remains constant over long periods; (c) high cost of short-run adjustments; (d) the presence of high fixed costs; (e) conflicts between farming being a business and a way of life; (f) limited knowledge of the economies and technology of farm production and even much misinformation. (15) Nevertheless, most farmers watched price movements carefully. R. W. Hunt

529. Pricing fat and skim components of milk. D. A. CLARKE, JR. and J. B. HASSLER, Cal. Agr. Expt. Sta., Berkeley. Bull. 737. 1953.

This bulletin discusses the difficulties of pricing milk in Cal., using M.S.N.F. as well as butterfat for the price fixing components.
R. W. Hunt

530. Agriculture and the people of our State, our Nation, and the World. M. F. MILLER, Mo. Agr. Expt. Sta., Columbia. Bull. 596. 1953.

This bulletin is written particularly for youth who are interested in world agriculture and also agriculture in the United States.
R. W. Hunt

531. Trends in European dairy plant equipment. O. WOLFRUM, Vienna, Austria. Milk Dealer, **43**, 6: 72. 1954.

The most revolutionary of the new trends in Austrian Dairying has resulted from the invention of the continuous butter making process and the introduction of the stainless steel

churn. A so-called cheese worker has been invented that makes it possible to produce large quantities of cheese rapidly, economically, and with minimum of manual work. Improved refrigeration also is reported. C. J. Babcock

532. Selling farmers dairy products. C. O. Davis, Jr. Milk Plant Monthly, **43**, 3: 12. 1954.

The Prairie Farms Creamery in Bloomington and Henry, Ill. is tapping the farm market for dairy products. It has a fleet of ten cream trucks doing a combination job. They pick up cream which is put in a rear compartment and kept under refrigeration at about 35°. In this same compartment, fresh concentrated milk is carried for retail distribution. Cottage cheese and powder also are carried. A front compartment is kept at from 10 to 20° below zero, and 380 gal. of ice cream can be carried for delivery to farm customers. The fresh concentrate is sold in qt. glas bottles and it has a butterfat content of 10½%.

The average farm housewife is buying 3 qt. of the concentrate per week — the equal of 9 qt. of milk. The most important thing to remember in moving into a farm market is to offer all the related dairy products which the farm family will want. C. J. Babcock

533. Inanimate cow milk dispenser. P. D. Mangano. U. S. Patent 2,670,102. 1 claim. Feb. 23, 1954. Official Gaz. U. S. Pat. Office, **679**, 4: 937. 1954.

A milk dispenser in a model of a cow. Raising and lowering the cow's tail, operates a plunger which delivers milk from a supply tank within the cow to a container held below one teat. R. Whitaker

534. Liquefied Petroleum Gas. W. J. Thompson, Carnation Co., Los Angeles, Cal. Milk Prod. J., **45**, 4: 26. 1954.

Liquefied petroleum gas has been used by this company since 1939. Various early problems have been solved and the operation is now considered highly successful.

Basically, there are 4 major differences between an engine equipped to burn liquefied petroleum gas and one equipped to burn gasoline: (1) it is desirable to use a higher compression ratio in order to get similar performance and economy from liquefied petroleum gas because of the lower b.t.u. content per gal. (2) a cold intake manifold without a "hot spot" is required for best performance with liquefied petroleum gas. (3) a gas-air carburetor is used in place of the liquid gasoline carburetor. (4) liquefied petroleum gas is a liquid only under pressure, hence a strong pressure tank is required. Other equipment consists of the necessary lines, valves, fittings, regulating valves and vaporizer. Engine ignition timing should be advanced about 10° to take full advantage of liquefied petroleum gas.

Certain precautions are necessary. Due to the pressure storage required, the gas must be carried in specially built, welded tanks of heavy steel which must be inspected and approved by a licensed state boiler inspector. The tanks must be built to conform to either the ASME or API-ASME codes and are designed with a minimum factor of safety of four times the maximum anticipated working pressure.

J. J. Janzen

FEEDS AND FEEDING

535. The relative feeding value for milk production of two National Cattle Foods. G. L. Bailey, W. H. Broster, B. B. Brown, and A. S. Foot, Natl. Inst. Research Dairying, Univ. of Reading. J. Dairy Research, **21**: 1. 1954.

National Cattle Food (N.C.F.) 1, in which the protein was mainly imported oilseed residues, was compared with N.C.F. 6, in which the protein came essentially from home grown artificially dried forage crops. Feeding trials were conducted using 42 first-calf heifers, mainly of Friesian and Shorthorn breeding. The N.C.F.'s supplemented hay in winter rations throughout 1951-52 and 1952-53 and contained approximately 20% crude protein. The animals had milk weights recorded each milking, and body weight periodically. N.C.F. 1 and N.C.F. 6 were mixed in ratios of 2:1, 1:1, and 1:2 and these rations were allotted randomly to animals each of which received certain quantities of hay with the balance of total requirements met by the mixture of N.C.F.'s. Animals were placed on experimental rations following calving, and data collected following an adjustment period. The average days in milk for the first and the second trial was 94 and 49 days. The input-output data was collected for one week in each season and daily means computed.

The formula used was: Milk yield (lb.) = hay (lb.) × b1 + N.C.F. 1 (lb.) × b2 + N.F.C. 6 (lb.) × b3 — liveweight (2/3) (lb.) × b4 in which the b coefficients were calculated by methods for multiple regression.

By comparing b2 and b3 the N.C.F. 1 was found to be but slightly superior to N.C.F. 6 when used with this group of heifers milking on an average approximately 29 lb. daily.

J. D. Donker

536. Vitamin B_{12} feed supplement. A. S. Hester, Assist. Ed., in collaboration with G. E. Ward, Dawe's Laboratories, Inc., Newaygo, Mich. Ind. & Eng. Chem., **46**, 2: 238. 1954.

Vitamin B_{12} is a complex cobalt-containing organic compound whose structure is not completely known. It is synthesized by a number of microorganisms. Its production has given rise to an important fermentation industry which produces vitamin B_{12} for supplementary use in swine and poultry feeds. Manufacture of the vitamin is discussed and is shown on a

flow sheet. The preferred fermentation organism is *S. olivaceus*. Air is filtered and medium and fermentors are sterilized. B. H. Webb

537. Winter feeding systems compared. Anon., Miss Agr. Expt. Sta., State College. 66th Ann. Rpt., 30. 1953.

Four different rations were fed: I, continuous grazing with no harvested roughage; II, grazing from 7:30 A.M. to 3:00 P.M. plus mixed alfalfa-Johnson grass hay fed free choice when not on pasture; III, grazing 7:30 A.M. to 3:00 P.M. plus silage free choice when not on pasture; and IV, barn fed control. Results showed when herbage was available, winter pasture of oats and crimson clover was an economical source of feed and contained a milk stimulating factor. R. W. Hunt

538. Whole cottonseed in dairy rations. Anon. Miss. Agr. Expt. Sta., State College. 66th Ann. Rpt., 30. 1953.

Two rations were fed to two groups of ten cows each. One ration consisted of equal parts of wheat bran, crimped oats and whole cottonseed, while the control ration was made up of 1 part wheat bran, 1 part crimped oats and 1 part of a mixture of 4 parts shelled corn and 3 parts expeller extracted cottonseed meal. No apparent difference in the palatability of the two rations was observed. As much as 5.5 pounds of whole cottonseed per day in the cottonseed ration were consumed. Significant difference favored the whole cottonseed ration in respect to both pounds of milk and pounds of butterfat produced per head. Both groups gained weight during the experiment.
R. W. Hunt

539. Antibiotic studies with dairy calves — Tyrothricin and Bacitracin. L. L. Rusoff and A. V. Davis, La. Agr. Expt. Sta., Baton Rouge. Proc. So. Agr. Workers, 76. 1952.

An antibiotic feeding supplement containing tryrothricin and one containing bacitracin were supplemented to a calf starter to determine what effect these antibiotics had on the growth of calves up through 90 days of age. Twenty-one purebred Holstein newborn calves were divided into three groups. Group I (control) received a 19.4% digestible protein calf starter containing no animal protein, group II — the calf starter supplemented with 2% tyrothricin feeding supplement (2 g. tyrothricin per lb. of supplement), and group III — the calf starter supplemented with 2% bacitracin feeding supplement (5 g. bacitracin per lb.). All calves were weaned from milk at 30 days of age, and were fed *ad libitum* the calf starter and medium quality clover hay beginning at 8 days of age.

The average daily gains for each group of calves at the end of 90 days were: I (control) 1.15 lb., II (tyrothricin) 1.22 lb., and III (bacithracin) 1.14 lb. The antibiotics fed did not appear to produce a growth stimulation in young calves, nor were any detrimental effects observed. Author's Abstract

540. Coarse grain or fine? N. F. Calovos and H. A. Keener, Univ. of New Hampshire, Durham. Guernsey Breeders' J., **90**, 6: 728. 1954.

Results are given from an experiment to determine if coarse feeds are more palatable to cows than good finely-ground feeds and if coarse feeds are digested and utilized as efficiently as fine feeds. The data indicated that high quality finely-ground dairy feeds were more digestible than coarsely-ground feeds. Differences in grain consumption between "fine" and "coarse" were not significant. Greater differences were noted between consumption of mixtures of the same formula than between coarse and fine grinding. A. R. Porter

GENETICS AND BREEDING

541. The influence of live weight on milk production during the first lactation. G. L. Bailey and W. H. Broster, Natl. Inst. Research Dairying, Univ. of Reading. J. Dairy Research, **21**: 5. 1954.

Records of heifers freshening in the period 1942-52 in the Dairy Shorthorn herd at Shinfield were arranged in groups according to sire. Seven sires with 99 daughters were used. Data collected were live weight at calving, age at calving, lactation length, and yield of 4% F.C.M. in a lactation. Using 4% F.C.M. as the dependent variate and live weight, age at calving and lactation length as independent variates, multiple linear regression equations were calculated. The data were treated as a whole and for each group of daughters by sire. Inheritance of size at first calving was measured by correlation coefficients provided by data from 47 dam/daughter pairs. This was not found to be significant.

Live weight and length of lactation both affected milk yield in a positive manner, and age at first calving did not affect production when comparing daughters of sires. Comparing sire groups, there was no effect on milk production from size, lactation length or age; meaning that daughters of bulls giving the most milk were not necessarily the largest animals within the total group.

Size was governed by environmental conditions within the breed in this herd; being favorable, large cows result, which have higher yield potential than their smaller sister animals.
J. D. Donker

542. Milk studied as semen diluter. Anon., Miss. Agr. Expt. Sta., State College. 66th Ann. Rpt., 31. 1953.

Provided a satisfactory and economical diluter for bull semen continues to be a major problem. Storage studies were made on homogenized, pasteurized whole, pasteurized skim and evaporated milk diluters with and without

antibiotics. Skimmilk was inferior to all the other milk diluters and homogenized milk was superior to the control yolk citrate diluter as well as all the other milk diluters.
R. W. Hunt

543. Dairy calves. Their development and survival. P. T. Dix Arnold and R. B. Becker, Fla. Agr. Expt. Sta., Gainesville. Bull. 529. 1953.

From 90 days to full term, male Jersey fetuses developed slightly faster than female. First calf Jersey heifers usually bore calves weighing about 5 lb. less than subsequent calves. Average gestation period was 277.9 days for 421 males and 276.5 days for 411 female Jersey calves. R. W. Hunt

HERD MANAGEMENT

544. Dairy labor requirements. S. E. Carpenter, Texas Agr. Expt. Sta., College Station. Prog. Rpt. 1621. 1953.

Labor involved in operating a milking herd of 31 Jersey cattle was recorded in units of minutes. R. W. Hunt

545. Animal operated stock oiler; and Livestock oiling apparatus. G. R. Rasmussen. U. S. Patents 2,669,968 and 2,669,969 respectively. 1 claim and 4 claims respectively. Feb. 23, 1954. Official Gaz. U. S. Pat. Office, **679**, 4: 899. 1954.

A flexible livestock oiling belt stretched from the top of a post to a stake a few feet away. Oil is admitted by gravity to the belt from a reservoir attached to the post by the action of the animal rubbing against the belt.
R. Whitaker

546. Weaner. C. H. Patterson. U. S. Patent 2,669,967. 1 claim. Feb. 23, 1954. Official Gaz U. S. Pat. Office, **679**, 4: 899. 1954.

A weaning device for attaching to the head of calves, etc., which is gravity-operated to provide access to the food, while the animal's head is down but closed if the head is lifted.
R. Whitaker

547. Teat cup for use in connection with milking machines. H. E. Skett. U. S. Patent 2,669,966. 3 claims. Feb. 23, 1954. Official Gaz. U. S. Pat. Office, **679**, 4: 899. 1954.

A milking machine teat cup is described consisting of a rigid outer shell, a flexible inner liner, and an outlet plug, which may be easily disassembled for cleaning. R. Whitaker

548. Continuous milk can filling attachment. H. A. Heckendorf (assignor to International Harvester Co.). U. S. Patent 2,670,889. 8 claims. March 2, 1954. Official Gaz. U. S. Pat. Office, **680**, 1: 184. 1954.

Milk from a milking machine line under vacuum, fills a series of milk cans automatically to a predetermined level. The flow into the cans is controlled by a float valve built into a TEE over each can. The valve construction is so arranged that the cans in the series are filled one at a time. R. Whitaker

549. Control unit for milking machines. A. G. Perkins. U. S. Patents 2,670,753 and 2,670,754. 10 claims and 18 claims respectively. March 2, 1954. Official Gaz. U. S. Pat. Office, **680**, 1: 143. 1954.

A device is described for releasing the milk from a vacuum milker to atmospheric pressure.
R. Whitaker

550. Inflation for milkers. J. J. Stampen (assignor to Babson Bros. Co.). U. S. Patent 2,670,709. 4 claims. March 2, 1954. Official Gaz. U. S. Pat. Office, **680**, 1: 131. 1954.

A description is given for a flexible liner for a teat cup for a vacuum milker.
R. Whitaker

551. Vacuum actuated door control for milking barns. H. H. Hill. U. S. Patent 2,671,428. 1 claim. March 9, 1954. Official Gaz. U. S. Pat. Office, **680**, 2: 397. 1954.

A milking barn having an entrance through which a cow enters to occupy a milking stall. After milking, the cow leaves through an exit door. Both doors are of the sliding type and are opened and closed by means of vacuum jacks. The controls for operating the doors, independently of each other, are located conveniently for the milking attendant. The doors may be opened wide for the cows or partially for admitting fresh air. R. Whitaker

552. Device for reversing resilient inflations used with milking machines. M. J. Gaver (assignor to Harry J. Lebherz, Jr.). U. S. Patent 2,672,063. 9 claims. Mar. 16, 1954. Official Gaz. U. S. Pat. Office, **680**, 3: 652. 1954.
R. Whitaker

553. Nipple for calf feeding buckets. M. J. McCrary. U. S. Patent 2,672,124. 1 claim. March 16, 1954. Official Gaz. U. S. Pat. Office, **680**, 3: 670. 1954.

A flexible nipple is attached to an outlet near the bottom of a pail. R. Whitaker

ICE CREAM

554. Low carbohydrate ice cream for diabetics. C. G. Roverts and C. D. Dahle, Pa. State College, State College. Ice Cream Rev., **37**, 8: 53. 1954.

Two mix formulas designed for the production of diabetic ice creams are presented. Mix No. 1 is calculated to contain 14% fat, 9% serum solids, 15% sorbitol, 0.2% C.M.C. as stabilizer and 0.02% saccharin, whereas mix No. 2 is calculated to contain 14% fat, 7% serum solids, 2% sodium caseinate, 13% sorbitol, 3%

glycerol, 0.1% sucaryl and 0.2% C.M.C. as stabilizer.

In mix No. 1, sweet cream and sodium caseinate were used to supply the fat and serum solids, whereas in the second mix cream, condensed skimmilk and sodium caseinate were employed to supply the milk solids. The caloric value per 100 grams portion was calculated to be 191.04 calories for mix No. 1 and 190.84 calories for mix No. 2.

Details of the information which should be included on the label of diabetic ice cream to meet legal requirements are presented.

W. J. Caulfield

555. Chocolate coated ice cream bars. R. A. SIMONET, Robt A. Johnson Co., Milwaukee, Wis. Ice Cream Rev., **37**, 8: 78. 1954.

Chocolate coated bars are a large production item with a narrow profit margin. Careful control and checking is essential to a profitable operation. Under ideal conditions a pound of coating should cover 38 3-oz. bars or 42 2½-oz. bars. The author offers numerous suggestions for achieving maximum efficiency in the coating operations. W. J. Caulfield

556. Machine-made milkshakes. ANON. Ice Cream Rev., **37**, 8. 50. 1954.

The introduction of a direct draw milkshake machine by the Mobley Ice Cream Co., in Ottawa, Illinois, has resulted in savings of $45 per week in labor costs and $20 per week in costs of freezing, hardening and tempering. This method of operation has eliminated the use of 40 5-gal. ice cream cans and two ice cream cabinets.

In the preparation of the milkshake, equal parts of homogenized milk and 6% custard mix are combined and frozen in the milkshake machine. Flavor is added to the serving container, the milkshake base is drawn directly from the machine into this container. A few seconds mixing assures uniform distribution of the flavor and the milkshake is ready to serve. Twenty-four-ounce milkshakes prepared in this manner sell at 35¢ each. Sales have averaged from 100 to 200 milkshakes per day which is an increase of over 30% in one year. An operator who sells 100 or more per day would find the use of a milkshake machine profitable.

W. J. Caulfield

557. Process control for ice cream. J. W. ROBERTSON, H. P. Hood & Sons, Boston, Mass. Ice Cream Trade J., **50**, 2: 54. 1954.

A system of using weights instead of volume and an accounting for all materials at the various stages of processing aid in finding what losses occur and where they occur in ice cream plant operation. Process control has made it possible to reduce shrinkage by 60% when 1951 figures are compared with 1953 results.

W. H. Martin

558. 1953 gallonage. Ice Cream Trade J., **50**, 2: 14. 1954.

According to the Bureau of Agricultural Economics, U. S. Dept. of Agriculture, ice cream production for 1953 was 606,550,000 gal., or 3% above the 591,576,000 gal. manufactured in 1952. In 1952 there had been a 4% increase above the 1951 figures. W. H. Martin

559. Sealtest markets a new flavor nationwide. Ice Cream Trade J., **50**, 2: 34. 1954.

A chocolate-almond combination flavor for ice cream called "Choc-o-nut" has been introduced by National Dairy Products Corporation.

W. H. Martin

560. Chocolate mix. G. C. NORTH, R. A. KLEIN, and P. P. NOZNICK (assignors to Beatrice Foods Co.). U. S. Patent 2,672,419. 1 claim. March 16, 1954. Official Gaz. U. S. Pat. Office, **680**, 3: 755. 1954.

Frozen concentrated milk is made by mixing milk with chocolate, sugar, and a stabilizer which imparts a low viscosity, pasteurizing for 10-30 min. at 155-185°, concentrating to 50-58% total solids, adding additional chocolate to obtain 52-62% total solids, cooling, and finally freezing the product. R. Whitaker

561. Apparatus for filling containers with ice cream and the like. F. C. GROSS and F. S. CHYTIL (assignors to Package Machinery Co.). U. S. Patent 2,672,262. 12 claims. March 16, 1954. Official Gaz. U. S. Pat. Office, **680**, 3: 710. 1954.

A machine for filling cartons with ice cream from the freezer, consisting of a nozzle which operates vertically with a reciprocating motion. When the nozzle rises from the lower position, a gate valve within the nozzle allows the ice cream to flow into the carton which surrounds the nozzle. At the top of the stroke, the valve closes and the nozzle is lowered into another empty carton to complete the cycle.

R. Whitaker

562. Method and apparatus for the extrusion filling of containers with ice cream and other similar semiplastic food substances. F. M. JOHANSEN. U. S. Patent 2,670,116. 21 claims. Feb. 23, 1954. Official Gaz. U. S. Pat. Office, **679**, 4: 942. 1954.

An ice cream extruder head for delivering as many as 6 streams of product into containers, molds, etc., from one supply line.

R. Whitaker

563. Apparatus for making variegated ice creams and the like. N. I. PEYTON (assignor to Joe Lowe Corp.). U. S. Patent 2,669,946. 4 claims. Feb. 23, 1954. Official Gaz. U. S. Pat. Office, **679**, 4: 893. 1954.

A device for continuously injecting a flavor-

ing material into ice creams as it leaves the freezer. Baffle plates are provided to insure uniformity. R. Whitaker

564. Means for striping and ribboning ice cream. C. W. WILDEBOUR. U. S. Patent 2,669,945. 7 claims. Feb. 23, 1954. Official Gaz. U. S. Patent Office, **679**, 4: 893. 1954.

A device for continuously injecting under pressure a stream of liquid flavoring material into ice cream as it leaves the freezer.
R. Whitaker

565. Merchandising machine for frozen comestibles. C. GABRIELSEN and W. R. POST (assignors to Rowe Manufacturing Co., Inc.). U. S. Patent 2,672,389. 6 claims. March 16, 1954. Official Gaz. U. S. Pat. Office, **680**, 3: 747. 1954.

A vending machine for individual servings of packaged ice cream, frozen novelties, etc.
R. Whitaker

MILK AND CREAM

566. High heat treatment of milk. R. DAVIS, Daveat Milk Processors Co., Inc., Philadelphia, Pa. Milk Dealer, **43**, 6: 49. 1954.

A high temperature, flash duration process, involving an entirely new principle of heat transfer is described. The principle is simple in design and practical in application, i.e., 1,000° heat, and higher, from an electric heating element radiates through air, not through metal, to a thin film of milk in a one-second drop down the inside cylinder surface of a machine. The cylinder metal behind the film of milk never becomes hotter than the milk itself, and therefore, "sticking on" does not occur. The flavor of the milk is reported to be equivalent to raw or properly pasteurized milk and, in many cases, superior. The destruction of bacteria is close to 100% and the equipment has excellent prospect for use in commercial sterilization. The cost of operation is considered to be comparable to pasteurization, depending on the conditions surrounding the particular plant operation. C. J. Babcock

567. Standards for cream quality. H. S. ADAMS, Ind. Univ. Med. School, Indianapolis. Milk Plant Monthly, **43**, 3: 29. 1954.

The control of cream quality used in the manufacture of ice cream starts on the farm where the milk is produced. Platform tests alone will not disclose substandard production methods and sanitary violations. While all of the detailed requirements established for Grade A fluid milk may not be as essential for milk for manufacture, there should be reasonable standards established and adhered to which will improve the quality of this grade of milk. A sound program of field supervision should be instituted. This should be educational in nature up to a point, but producers who demonstrate no intention of meeting minimum requirements should be excluded from the market. Regular testing of each producer's milk should be done by an industry and with the advice and assistance of regulatory agencies. There is a real need for demonstrated leadership in this phase of dairy production and while obstacles may loom large, they are not unsurmountable.
C. J. Babcock

568. A study of the influence of agitation time on the Babcock test of milk samples from farm bulk holding tanks. B. L. LISKA and H. E. CALBERT, Dept. of Dairy and Food Ind., Univ. of Wis., Madison. J. of Milk and Food Technol., **17**, 1: 14. 1954.

Mechanical stirring of the milk in the farm bulk milk holding tanks for 60 sec. was satisfactory for proper sampling for the butterfat test.

The procedure was satisfactory where various volumes of milk and different creaming times were involved. H. H. Weiser

NUTRITIVE VALUE OF DAIRY PRODUCTS

569. The nutritive values of fat. R. T. HOLMAN, Univ. of Minn., Austin. Milk Plant Monthly, **43**, 3: 33. 1954.

Fat yields the greatest amount of energy per unit weight of any of the 3 basic constituents of food. Fat storage is the most concentrated form of energy storage in the animal. A reasonable amount of fat is beneficial by virtue of its high heat content, the indirect effects it has upon digestion and absorption of other nutrients, and the specific nutritive requirements it supplies. By these standards butterfat is a very important item in the national diet and it fulfills most of the nutritive functions of fat. C. J. Babcock

PHYSIOLOGY AND ENDOCRINOLOGY

570. Intramammary pressure changes in the lactating sow. I. The effect of oxytocin. G. W. WHITTLESTONE, Ruakura Animal Research Sta., Dept. Agr., New Zealand. J. Dairy Research, **21**, 1: 19. 1954.

The sow was chosen to study intramammary pressure response to oxytocin as this animal does not release oxytocic substances which interfere with injected doses except under the influence of suckling pigs. Further, the mammary gland sinus and the duct system have small capacity and therefore the pressures within the gland could be more precisely measured. An electronically recording mercury-saline manometer with a small volume displacement was devised and proved very satisfactory. Observations following injections indicate that contractions are not long maintained but they may be reinitiated after a brief refractory period of about 15 to 20 sec. Following a single injection

of 0.5 i.u., quite often a secondary response was seen, which was thought to coincide with recirculated oxytocin returning to the udder. When using a graded series of doses from 0.1 i.u. to 0.6 i.u., the pressures recorded ranged from 14.5 to 26 mm. Hg and the duration of response lasted from 15.8 to 25 sec. There appeared to be a positive relationship of both these measurements to level of dose administered but pressure relationships were more consistent in their relationship to various amounts injected. A figure illustrates the electro-manometer used and there is an illustration of the circuits concerned with the amplifier and recording system used. J. D. Donker

571. The biosynthesis of protein. I. Uptake of glycine, serine, valine and lysine by the mammary gland of the rabbit. P. N. CAMPBELL and T. S. WORK, Natl. Inst. for Med. Research, London. Biochem. J., **52**, 2: 217. 1952.

Radioactive glycine, valine and lysine were injected into rabbits and followed during the formation of milk proteins. From activity of the plasma protein as compared to milk protein, it was concluded that most of the nitrogen of milk proteins comes from free amino acids from the blood rather than from peptides.

There are 5 tables, 5 figures and 71 references. Much space is devoted to a discussion of the literature and descriptions of methods and materials. A. O. Call

572. Anaerobic and aerobic glycolysis in lactating mammary gland and in nervous tissue. C. TERNER, Univ. of Reading. Biochem. J., **52**, 2: 229. 1952.

The metabolism of glucose in mammary gland slices and in nervous tissue is reported. Under certain conditions 2:4-dinitrophenol does not influence glucose breakdown or lactic acid formation in a parallel manner. Results are reported in 11 tables and there are 46 references. A. O. Call

SANITATION AND CLEANSING

573. In-place cleaning of milk pasteurizing equipment. A. V. MOORE and F. E. POTTER, Texas Agr. Expt. Sta., College Station. Bull. 770. 1953.

The cleaning in-place of S.S. milk lines, using acid and alkaline rinses, and sanitizing them with chlorine, is a satisfactory procedure. In-place cleaning and sanitizing of a plate type regenerator-pasteurizer-cooler is satisfactory if, following the water-acid-water-alkali rinse sequence, the plates in the unit are inspected and lightly brushed or hosed with cold water. R. W. Hunt

574. Industrial glass piping. Evolution and present application. E. B. SHAND, Corning Glass Works, Corning, New York. Ind. & Eng. Chem., **46**, 1: 179. 1954.

The development of industrial glass piping during the last 25 years is reviewed. Performance of the present product differs greatly from that of the early piping. Industrial glass piping with accessory fittings is available in sizes from 1 to 6 inches. Stainless steel shortages during the war focused attention on use of glass piping in milk plants. The current revision of the U.S.P.H.S. sanitary code recognizes in-place cleaning of pipelines for fluid milk, so the way is now open for increased use of glass pipe in dairy plants. B. H. Webb

575. Modern wash facilities. ANON. Ice Cream Rev., **37**, 8: 47. 1954.

Providing employees with adequate facilities for assuring personal cleanliness is important in reducing possible contamination of the product and is equally important in impressing employees with the importance of personal cleanliness in maintaining acceptable quality standards in a food processing plant.

The Borden Food Products Co. has found it desirable to have the locker room and toilet facilities adjoin each other with a connecting door. Every employee leaving the toilet must pass directly by a wash fountain. Such an arrangement makes the hand-washing facilities easy to use and serves to remind employees that their hands should be washed.

Wash fountains have proved more satisfactory than numerous individual lavatories. Each fountain will serve 8 to 10 people simultaneously and yet require only two supply connections and one drain, thus markedly reducing installation costs. Water leakage is reduced by 95% and there is only one drain and trap to get out of order and only one soap dispenser to provide. In addition, time needed for keeping the wash fountain clean is much less than with the conventional lavatory, and the wash rooms are kept neater since splash is virtually eliminated. W. J. Caulfield

576. Biological studies of horse flies in New York. H. TASHIRO and H. H. SCHWARDT, Cornell Univ., Ithaca, New York. J. Econ. Entomol., **46**, 5: 813. 1953.

Biological studies of horse flies attacking dairy cattle were made in New York. Forty-eight species of horse flies were collected. Seventy-six % of those collected were of five species: *Tabanus quinquevittatus, T. lasiophthalmus, T. sulcifrons, T. pumilus,* and *T. lineola;* the first two species constituted 58%. *Tabanus* sp. were much more important than *Chrysops* sp. as pests of cattle.

Seasonal abundance of horse flies varied with weather and species. Peak population periods varied from about 1 to 3 weeks. Earliest horse flies appeared in late May, and latest ones were collected during the second week of September of another year.

Horse flies attacked cattle which were: of poor health more than healthy ones, larger

animals more than the smaller, and dark-colored ones more than those light-colored. Possible blood loss per cow was estimated at 352 cc. per day, due to a particular species during peak flight. Two species of non-blood sucking flies lapped up blood oozing from horse fly punctures, becoming a nuisance to the cattle.

Large numbers of horse fly larvae were found in pasture sod where a semi-hydrophytic plant, *Carex hystericina*, grew well. A maximum of 27 larvae of one species was found in one square yard sample. Most larvae were in the upper 3 to 4 inches of soil. E. H. Fisher

577. A comparison of evaporation rates and toxicity to house flies of lindane and lindane-chlorinated polyphenyl deposits. C-H Tsao, W. N. Sullivan, and I. Hornstein, Bureau of Entomology and Plant Quarantine, Agr. Research Adm., U.S.D.A. J. Econ. Entomol., **46**, 5: 882. 1953.

Laboratory tests showed a chlorinated polyphenyl to prolong the residual effect of lindane in house fly control. The rate of lindane evaporation was retarded by the chlorinated polyphenyl, and there was an indication that it synergized lindane. E. H. Fisher

578. Concentrations and exposure time of lindane vapor required to kill insects. W. N. Sullivan and I. Hornstein, U.S.D.A., Agr. Research Adm., Bureau of Entomology and Plant Quarantine. J. Econ. Entomol., **46**, 5: 898. 1953.

Lindane vapor was tested in the laboratory against five species of insects. The house fly, yellow-fever mosquito, and bean weevil were effectively controlled with 0.60 to 1.19-γ of lindane per l. of air in periods ranging from 2 to 30 min. It was concluded that a higher concentration and longer exposure would be necessary to control the American cockroach and confused flour beetle. E. H. Fisher

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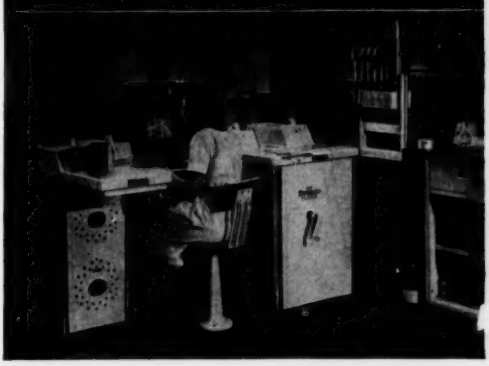

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